Population ecology of the red grouse, Lagopus lagopus scoticus, with particular reference to the effects of the parasite trichostrongylus tenuis

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Population ecology of the red grouse, *Lagopus lagopus scoticus*, with particular reference to the effects of the parasite *Trichostrongylus tenuis*

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Declaration

The material contained in this thesis has not previously been submitted for a degree at the University of Durham or at any other university. The research reported in this thesis has been conducted by the author, unless otherwise indicated.

Population ecology of the red grouse, *Lagopus lagopus scoticus*, with particular reference to the effects of the parasite *Trichostrongylus tenuis*.

ABSTRACT

Trichostrongylus tenuis is a prevalent nematode parasite of red grouse *Lagopus lagopus scoticus*. It reduces breeding success and survival of birds, and as a result may be responsible for the cycles in bird density that occur in many red grouse populations. In this thesis I examine this host-parasite interaction, including testing the effectiveness of parasite control, studying the frequency distribution and spatial distribution of parasites in a grouse population, and investigating the nature of parasite-induced cycles in host numbers through theoretical modelling.

Many grouse moors rely on the application of anthelmintic-coated grit for controlling nematode infection in red grouse. This grit is placed on the moor for the grouse to consume, which they do to aid digestion. However, a possible side effect of frequent dosing is the development of parasite resistance to the anthelmintic. I tested for resistance in parasites from 12 different moors in northern England, which varied in the timing of grit treatment and the quantity of grit applied to the moor. Egg hatch assays on *T. tenuis* eggs showed no evidence of resistance on any of the moors.

Studying the spatial distribution of parasites in the environment, and the degree to which they coincide with the spatial arrangement of the hosts, is fundamental to understanding the host-parasite interaction. A detailed survey of the distribution of *T. tenuis* on an area of moorland in Teesdale, northern England, supported the hypothesis that the parasite population is not uniformly distributed among the host population: both adult parasites among hosts and eggs among caecal faeces were aggregated. Environmental factors and host characteristics played a role in determining the parasite distribution, with parasite infections being associated with age of birds and location on the moor. However *T. tenuis* egg concentration in caecal faeces on the moor was only weakly spatially auto-correlated suggesting that further intrinsic or extrinsic factors may be influential. Distribution of eggs on the moor was not related to the density of grouse.

Finally, I developed an individual-based stochastic model, which specifically modelled the red grouse-*T. tenuis* interaction. This showed that the parasite could theoretically cause cycles in grouse abundance, with the spatial distribution of both the host and parasite being important in the occurrence of cycles. Adding density-dependent host mortality to the model, had a stabilising influence on the host population, although the parasite still generated cycles in host numbers. In some cases this density dependence generated damped cycles in host numbers in the absence of the parasite. These cycles were amplified when parasite induced mortality was included, suggesting that the parasite can increased the cyclic tendency of the host population in these cases. Cycle periods were influenced by parasite-related parameters and were similar to those recorded in natural grouse populations.

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CHAPTER 1

General introduction

1.1 Host parasite interactions

Conservation and management strategies for individual bird species frequently require an understanding of population dynamics, and detailed studies of the factors that influence the size of the population and how these vary temporally and spatially (Dobson & Hudson 1995). One factor that can potentially cause changes in population numbers is parasite infection. Many studies have demonstrated that parasites can have a detrimental affect on host fecundity and survival in wild animal populations (e.g. for review see Tompkins & Begon 1999). One important question in wildlife epidemiology is whether parasites can influence host population dynamics and whether they may regulate host populations (e.g. Grenfell & Dobson 1995; Anderson & May 1978; Scott & Dobson 1989).

Red grouse *Lagopus lagopus scoticus* have been the subject of intensive study in Great Britain. Most birds are infected with the nematode parasite *Trichostrongylus tenuis*, which has a detrimental effect on grouse breeding and survival. In addition, the parasite can regulate the host population, and there is growing evidence that it may be a significant factor in the occurrence of population cycles in the host. Failure to understand the cause of grouse population cycles has been a major constraint on the efficient management of grouse estates in Britain (Dobson & Hudson 1994). The role of the parasitic nematode in the population dynamics of red grouse populations is thus of practical use in the management of many upland estates.

1.2 Biology of the red grouse

1.2.1 Red grouse distribution and management

Red grouse inhabit the heather moorlands of the British Isles. Their distribution is influenced by heather (*Calluna vulgaris*), their main food source (Savory 1978), and they are rarely found on other types of vegetation (Hudson & Watson 1985). Populations are managed to maintain a sustainable harvest of birds for sport shooting; in this respect they are unlike lowland game birds such as partridge and pheasants, which are reared in captivity before being released for shooting (Hudson & Watson 1985). Grouse moorland management involves the maintenance of the heather habitat through controlled burning and grazing, the control of predators, in particular foxes, crows and

stoats, and the control of parasites and disease such as louping ill (for review see Hudson & Newborn 1995). The productivity of grouse moors varies across Great Britain. Moors in England tend to have higher densities of grouse than those in Scotland (Hudson 1992). Drier moors in the east support high densities of grouse (50 pairs km^{-2}), while others (e.g. western Ireland) can have as few as 2.5 pairs of grouse km^{-2} (Hudson & Watson 1985).

1.2.2 Red grouse life cycle and behaviour

Red grouse are typically monogamous and territorial (Figure 1.1). Territory size and territorial aggressiveness vary according to bird density (Watson *et al.* 1994; Moss *et al.* 1996), with territories as small as 1 hectare being recorded at high density (Moss & Hudson 1990). Birds that do not obtain territories tend to emigrate or die before the breeding season (Jenkins *et al.* 1963; Hudson 1992; Watson *et al.* 1994). Hens lay an average clutch of 8 eggs in spring, which hatch in May (Hudson 1986a). After hatching many parents and young stay together at first but eventually family groups break up. Young hens disperse farther than cocks, but dispersal of more than 5km is infrequent in both sexes (Jenkins *et al.* 1967). Life expectancy of a grouse is 2 years (Hudson 1986a).

Shooting forms a significant income to many upland estates (Hudson & Dobson 1989), since approximately 100-400 brace (pairs of birds) can be shot in one day (Hudson & Dobson 1989). There are approximately 460 grouse moors in Great Britain, with an annual grouse shooting of approximately 450 000 grouse (250 000 of this in Scotland) (Hudson 1992). Most grouse shooting occurs in the first six weeks of the season, which runs from August 12^{th} to December 10^{th} (Hudson & Newborn 1995). Moors usually shoot between 30% and 50% of their birds depending on the density and conditions (Hudson & Newborn 1995). Old male birds are most likely to be shot (Hudson & Watson 1985) because they break away from family groups to begin forming territories in Autumn (Jenkins *et al.* 1967) and are more likely to fly during shoot drives. Territorial cocks will be replaced by non-territorial individuals whose chances of survival increase when they obtain a territory (Jenkins *et al.* 1963). Shooting loss can therefore be compensated for, to some extent, by an increase in the survival chances of remaining birds (Hudson & Watson 1985).

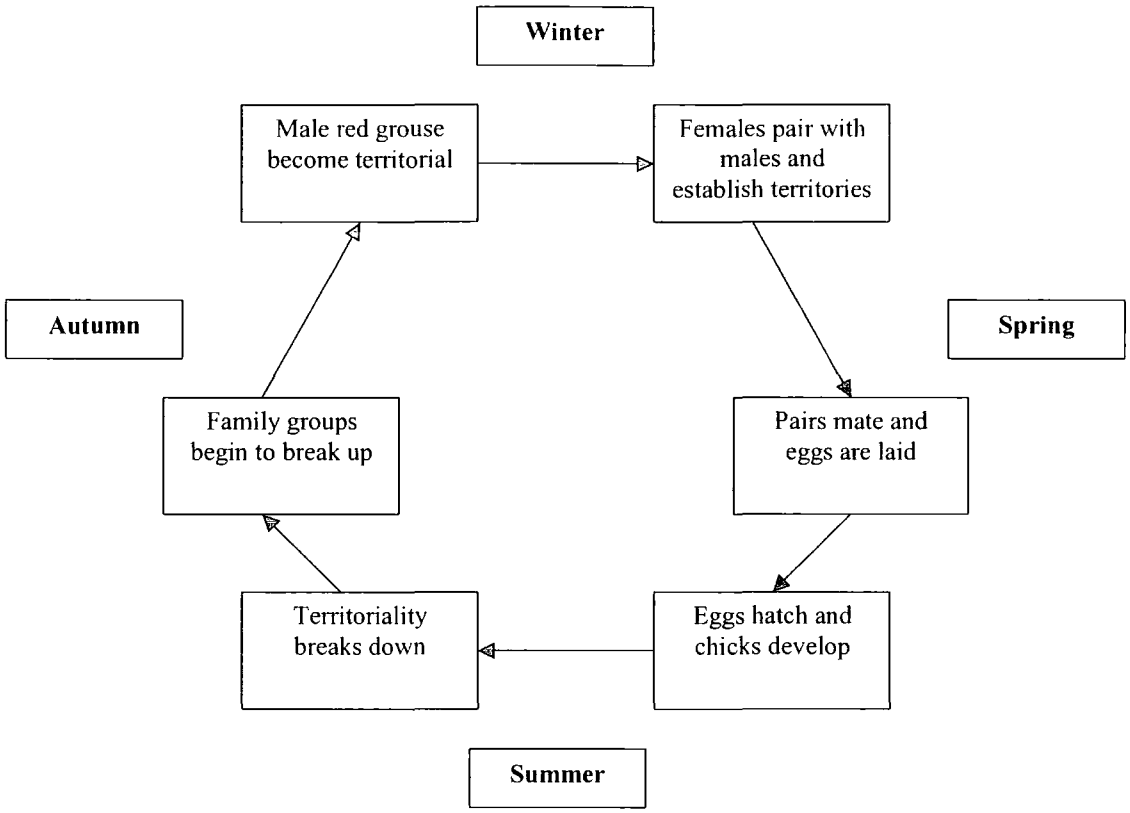


Figure 1.1 The life cycle of the red grouse

1.3 Red grouse and parasites

1.3.1 The red grouse and the nematode parasite *Trichostrongylus tenuis*

Red grouse are infected with the nematode parasite *Trichostrongylus tenuis*. It is extremely prevalent with more than 90% of birds infected (Wilson 1983; Hudson 1986a). Infections can be intense: as many as 30000 adult parasites in one bird have been recorded (Wilson 1983). *T. tenuis* tends to be the only nematode found in red grouse. Other common internal parasites include tapeworms (*Davainea urogalli*) in the small intestine (Jenkins *et al.* 1963; Shaw & Moss 1989a; Delahay 1999), and less commonly coccidia (Jenkins *et al.* 1963). The birds have little or no immunity against *T. tenuis* (Wilson & Wilson 1978; Hudson 1992, Shaw and Moss 1989b) and parasite burdens increase as a bird ages (Wilson 1983; Potts *et al.* 1984; Hudson *et al.* 1985; Shaw & Moss 1989b). Adult *T. tenuis* can survive more than 2 years in captive grouse

with little mortality (Shaw & Moss 1989b). Survival is high compared to other *Trichostrongylus spp.*, which tend to survive 3 to 4 months in grazing animals (Dunn 1978).

T. tenuis inhabit the caecae of a range of avian hosts such as the bobwhite quail *Colinus virginianus* (Moore *et al.* 1986; Davidson *et al.* 1991) and the domestic chicken *Gallus domesticus* (H. Watson *et al.* 1988). In these species, however, there appears to be spontaneous recovery from infection, with hosts expelling an established infection in a few weeks. The prevalence and intensity of infection in red grouse is high compared to other species (H. Watson *et al.* 1988). The difference may be related to a relatively poor protein diet compared with other hosts (Hudson & Dobson 1997). Within the habitat of red grouse other potential hosts are generally absent, and grouse are therefore considered to be the only host for *T. tenuis.*

1.3.2 Life cycle of *T. tenuis*

T. tenuis is a direct life cycle nematode (figure 1.2). Adult parasites inhabit the two blind-ended caecae at the end of the birds' gut. They reproduce here and eggs pass from the bird in the caecal faeces (brown glutinous droppings which contrast with the more typical fibrous droppings) (Hudson & Newborn 1995). Survival and development of eggs through two larval stages to infective third stage larvae depends on moisture (Watson 1988; Shaw *et al.* 1989) and temperature (Watson 1988; Shaw *et al.* 1989; Connan & Wise 1993, 1994). In optimum conditions, development from egg to third stage larvae (L3) can take 9 days, although eggs can remain unhatched for several months (Shaw *et al.* 1989; Connan & Wise 1993, 1994). Third stage larvae migrate from the caecal faeces to the growing tips of the heather (McGladdery 1984; Saunders *et al.* 1999) and grouse become infected when they feed. Larvae that successfully reach the gut caecae develop via a fourth stage, into sexually mature adult worms in as little as 12 days (Shaw 1988), although development of larvae can be arrested for several months after ingestion (Hudson 1986a; Shaw 1988). Factors causing arrestment are not clearly understood although it may synchronise parasite development with events in the host and environment (Gibbs 1986). Greatest numbers of arrested larvae have been found in grouse during winter (Shaw 1988).

1.3.3 Timing of parasite recruitment

Host parasite infection can vary annually and seasonally. In a study in northern England, variations between years in the size of the summer infection were positively correlated with grouse density in July of the previous year and with minimum July temperature (Hudson *et al.* 1992b). Studies in Scotland, however, have found that rainfall in previous summers explained much of the year to year variation in egg counts, probably because parasite recruitment was greatest during wet summers (Moss *et al.* 1993b)

Within a year, infection of grouse with *T. tenuis* varies seasonally. Large increases in parasite infection sometimes occur in February or March (Hudson *et al.* 1992b, Moss *et al.* 1993b). This winter infection may occur through direct infection of larvae, which are able to survive winter conditions (Connan & Wise 1993; 1994) and/or possibly due to larvae that entered a period of arrestment in autumn, before resuming development in February (Hudson 1992). However hypobiotic (arrested) larvae have not been found in numbers great enough to explain the very large increases in adult worm burdens in spring (Connan & Wise 1993). Gradual increases occur in summer as infective larvae are picked up from heather and mature (Hudson *et al.* 1992b). In autumn and winter the rate of larval establishment reduces (Hudson *et al.* 1992b; Moss *et al.* 1993b) since free-living *T. tenuis* eggs and larvae are susceptible to harsh environmental conditions (Connan & Wise 1993, 1994).

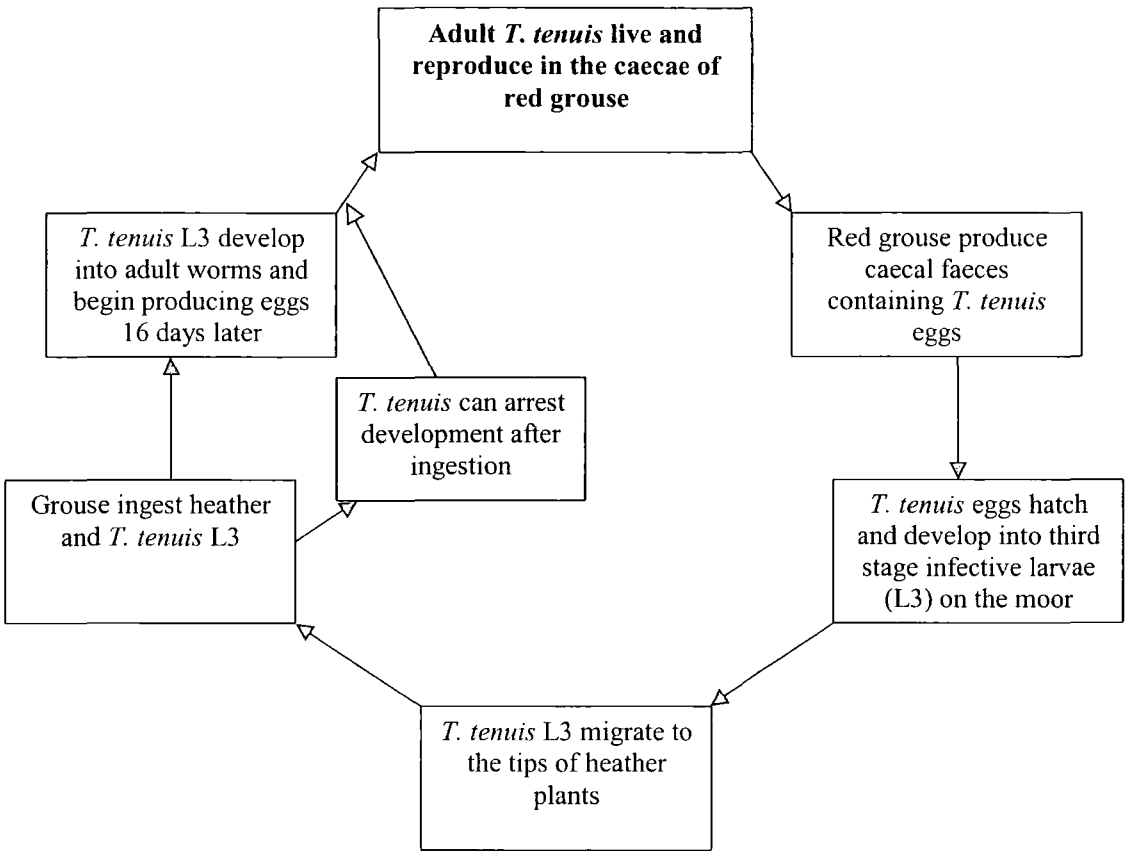


Figure 1.2 The life cycle of *Trichostrongylus tenuis*.

1.3.4 The impact of *T. tenuis* on red grouse

T. tenuis has a significant sub-lethal impact on the red grouse host (for review see Hudson & Dobson 1991) although it can be lethal when worm intensity is high (Wilson & Wilson 1978). Although the impact of *T. tenuis* increases with parasite intensity, there is not a clear relationship between the number of adult worms in a bird and its condition. (Jenkins *et al.* 1963). A decline in body condition is apparent in grouse carrying more than 3000 worms (Hudson 1986a). It has been suggested that the greatest effect of the parasite appears to be while larvae are developing into adults (Shaw & Moss 1990; Delahay *et al.* 1995).

Adult *T. tenuis* burrow into the lining of the gut caecae, where they cause severe internal bleeding and interfere with metabolism and digestion of heather (Watson *et al.* 1987).

The parasite can reduce body weight and condition of individuals (Wilson & Wilson 1978; Shaw & Moss 1990; Hudson 1986a, b; Delahay *et al.* 1995). This has a number of knock-on effects. In particular, parasite infection has a detrimental influence on breeding success. It interferes with egg production by causing a reduction in feeding and a consequent detrimental effect on body weight of females (Shaw 1990; Delahay & Moss 1996). In addition, captive hen red grouse infected with *T. tenuis* start to lay later in spring at a slower rate (Shaw & Moss 1990) and lay fewer eggs than uninfected hens (Wilson 1979; Shaw & Moss 1990). Treatment to reduce worm burdens in wild red grouse resulted in an increase in the number of chicks produced per female (Hudson 1986b; Newborn & Foster 2002). Developing larvae seem to have a stronger effect on red grouse egg laying than do adult worms that are already established in the bird (Shaw & Moss 1990; Delahay & Moss 1996).

Furthermore, *T. tenuis* can influence physiology and behaviour of individuals. Grouse infected with many parasites may be more vulnerable to predation. Prior to incubation, hen grouse stop producing caecal faeces and therefore scent emission decreases. This reduction is thought to be an adaptation to reduce detection by predators (Hudson *et al.* 1992a). There is some evidence that the parasite hinders the control of scent emission and so birds with high parasite burdens are more vulnerable to mammalian predation (Dobson & Hudson 1995).

Grouse with heavy parasite burdens can be less aggressive and therefore less able to compete for territories (Jenkins *et al.* 1963). These birds are forced to feed in areas where food quality is poor and are less likely to survive than territorial individuals (Jenkins *et al.* 1963). Grouse treated to reduce parasite burdens can become more aggressive and win more territorial contests (Fox & Hudson 2001). When male grouse have been implanted with testosterone to increase aggression, they increased their territory size and overall the population density was reduced (Moss *et al.* 1994; Mougeot *et al.* 2003).

1.3.5 The treatment of red grouse to reduce *T. tenuis* infection

Practical techniques have been developed to treat red grouse with anthelmintics to reduce the *T. tenuis* burdens and consequently increase survival and improve breeding success (e.g. Hudson 1986a, Hudson *et al.* 1992b; Newborn & Foster 2002). There are two methods of treating birds. Grouse can be caught at night and treated directly with an oral anthelmintic (commonly used liquid drenches such as Oramec (ivermectin) or Nilverm (levamisole hydochloride) commercially available for treatment of domestic livestock). Although the 'catch and treat' technique is labour intensive (approximately 50 birds can be treated per hour (Hudson & Newborn 1995)), it provides immediate reduction in parasites. The caecae of birds with small parasite burdens (2000-3000 worms) show some recovery after treatment, which can last 5 to 6 months (Watson *et al.* 1987). However re-infection can occur soon after treatment and within a year the effects on the worm population are likely to be quite small (Hudson 1992). It would be impractical to reduce the parasite burdens to a level where they had no significant impact on grouse breeding since at least 65% of the population would need to be treated (Hudson 1992). The technique is therefore most useful when a parasite outbreak is expected or has begun (Hudson 1992).

Red grouse can also be treated indirectly, by providing grit coated with anthelmintic ('medicated grit') on the moor for the birds to consume. Indirect parasite treatment where the drug is incorporated into an animals feed has been used in various systems (for example in the control of gapes, a nematode infection in pheasants (Hudson & Rands 1988)). Grit is used by grouse in digestion, to break up the fibrous heather in the gizzard (Hudson & Newborn 1995); without it grouse body condition can deteriorate (Hudson 1992). Medicated grit is regularly eaten by grouse, even when there are natural supplies (Hudson & Newborn 1995). Grit piles are placed at regular intervals on the moor, providing several piles in each grouse territory (Hudson & Newborn 1995). Grit provisioning occurs in the autumn and winter months to prevent denaturing of the active ingredient during the warmer months, and to ensure that grouse are not carrying the active drug within 24 days of August 12th. Although it is unlikely that all grouse consume medicated grit, medication reduces parasite burdens (Hudson 1992, Newborn & Foster 2002) and increases the number of chicks surviving (Newborn & Foster 2002).

One potential consequence of the use of anthelmintics is the development of parasite resistance, which could have important consequences for host-parasite population dynamics.

1.4 Monitoring red grouse populations

Red grouse populations have been monitored for over a century in the UK, with shooting records dating back to the 1830s. The numbers of birds shot (termed the grouse bag) are affected by factors such as weather and harvesting effort. The number of days harvesting tends to increase with grouse abundance (Hudson *et al.* 2002) and shooting generally stops when bird density is less than 30 birds km^{-2} (Hudson *et al.* 1998). However grouse bags are related to the density of individuals counted before harvesting, and hunting records are considered a good indicator of grouse abundance (Cattadori *et al.* 2003).

In general, numbers of grouse shot has declined over the past century (Hudson 1986a; Barnes 1987). Reasons for the decline depend on the geographical area and include loss and deterioration of habitat, overgrazing by sheep, poor standards of heather burning, the spread of sheep ticks and the louping ill virus, parasites and over shooting (Barnes 1987; Moss & Hudson 1990). As well as the long-term decline, analysis of red grouse time series has shown that many red grouse populations cycle in numbers with periods of 3-4 years (Williams 1985; Potts *et al.* 1984; Hudson 1992; Hudson *et al.* 2002), and up to 15 years (Hudson 1992; Haydon *et al.* 2002). The amplitude of the cycles varies from 2 fold up to 10 fold (Moss & Watson 2001). Red grouse cycles are generally 'phase forgetting quasi-cycles' (Nisbet & Gurney 1982), that is, the cycle amplitude dampens over several years and peaks occur at irregular intervals.

Many species show fluctuations in population numbers periodically over time with a statistically significant tendency for fluctuations to be repeated at non-random intervals. Periodic fluctuations in animal populations were first noted by Elton (1924). Recent analysis of 694 time series (220 species) showed that 30% exhibited periodic oscillations (Kendall *et al.* 1998). Examples include lynx (*Lynx canadensis*) (Elton & Nicholson 1942), snowshoe hare (*Lepus americanus*) (Krebs *et al.* 1995), small

mammals such as field voles (*Microtus agrestis*) (review in Krebs & Myers 1974) and birds of the grouse family (Tetraonidae) including the red grouse (Lindstrom *et al.* 1995; Moss & Watson 2001).

Population cycles are generally caused by a density-dependent regulatory effect acting with a time delay (May 1981). If the proportional loss from a population increases with density then the numbers will be stable, but when such effects act with a time delay there is a tendency for the numbers to cycle. For example some populations of snowshoe hare (*Lepus americanus*) show cycles in numbers with a period of about 10 years. One explanation for the cycles is that as numbers build up, predators such as lynx (*Lynx canadensis*) feed on the abundant prey. When the population begins to decline the ratio of prey to predators decreases and so the proportion of hares eaten by predators increases (Keith & Windberg 1978). Predation pressure on the hare population in any year is therefore related to hare density approximately two years earlier.

There have been many alternative explanations for population cycles, including interactions with predators, parasitoids, pathogens, food quality and quantity or genetic effects (Lotka 1925; Volterra 1926, Nicholson & Bailey 1935; Rosenzweig and MacArthur 1963; Anderson & May 1978; Matthiopoulos 1998; Schofield *et al.* 2002). A few long-term experimental manipulations of wild populations have identified density-dependent factors regulating population size. These include predator and food manipulations in hares (Krebs *et al.* 1995), and manipulations of density (Moss *et al.* 1996) and parasites (Hudson *et al.* 1998) in red grouse. These experiments can produce very useful data, although they can be difficult to replicate sufficiently (for review see McCallum 1995). A powerful complimentary method is to use mathematical population models to make quantitative predictions about a population's response to manipulation. This method makes it possible to investigate which factors are potentially capable of generating cyclic dynamics. Although it is impossible to include all population variables, cyclic populations are particularly amenable to modelling because relatively few interactions dominate the system (Kendall *et al.* 1999).

In red grouse there are a number of factors that could act in a delayed density-dependent manner to cause cycles in abundance. A range of hypotheses has been generated involving the effects of food, predation, shooting, spacing behaviour of the birds and the effect of the parasite *T. tenuis*. At present only two theories are considered to be strong hypotheses. First, work in Scotland has concentrated on changes in spacing behaviour influencing aggression and consequently density. Second, research in northern England has focused on the effects of the parasitic nematode *T. tenuis*. Other processes, such as predation and food quality, might interact with these regulatory mechanisms to shape the observed dynamics, but are not thought to cause cycles (Moss & Watson 2001; Thirgood *et al.* 2000). These hypotheses are described below.

Spacing behaviour

The kin facilitation hypothesis suggests that the effects of differential aggression between kin and non-kin, influence territory size and could caused cyclic variations in the recruitment of young males into the breeding population (Mountford *et al.* 1990; Moss & Watson 1991; Watson *et al.* 1994; Matthiopoulos *et al.* 2000).

In summary the hypothesis is as follows. Red grouse territorial aggressiveness limits density by affecting territory size (Moss *et al.* 1994). Related males tend to form territories close to one another and so clusters of related territorial males can build up (Watson *et al.* 1994; MacColl *et al.* 2000). In addition territorial males have fewer boundary disputes with related than with unrelated neighbours (Watson *et al.* 1994). Individuals tolerant of related neighbours will take smaller territories, and therefore on average produce relatively more total offspring per unit area. Recruitment of relatives into territorial clusters is allowed through kin tolerance and reduction of average territory size. When density reaches a peak, territory sizes approach the minimum and kinship cluster sizes can no longer be maintained. The chance of an old male that dies being replaced by a son decreases. As a result the average relatedness of neighbours decreases and causes a population decline through increased aggressiveness and reduced recruitment. Low recruitment rate maintains small kinship clusters, which in turn maintain high levels of intolerance. Individual territory size therefore increases and

population density decreases. Eventually population density falls to such a low level that recruitment rate and therefore density, increases again.

Theoretical modelling supports this hypothesis (Mountford *et al.* 1990; Hendry *et al.* 1997; Matthiopoulos *et al.* 1998, 2000, 2003) and experimental manipulation in wild grouse populations has demonstrated that intrinsic mechanisms can affect the population dynamics. For example, removing some males during the increase phase of the cycle prevented a subsequent cyclic decline Moss *et al.* (1996). More recently experimentally increasing aggressiveness for a short period of time in autumn reduced recruitment and subsequent breeding density and changed population trajectories from increasing to declining (Mougeot *et al.* 2003).

Parasitism

Anderson & May (1978) and May & Anderson (1978) demonstrated theoretically a number of aspects of a parasite system that could destabilise host numbers and generate population cycles. Parasites have been shown to regulate host populations through experimental manipulations of a few wild species (e.g. reindeer *Rangifer tarandus*, Albon *et al.* 2002) and have been implicated in some host population cycles (e.g. Soay sheep *Ovis aires*: Gulland 1992; snowshoe hares *Lepus americanus*: Ives & Murray 1997). *T. tenuis* can have a detrimental effect on survival (Wilson & Wilson 1978; Hudson *et al.* 1992b) and can reduce the breeding success of grouse (Potts *et al.* 1984, Hudson 1986b; Hudson *et al.* 1992b). If parasites reduce host fecundity or survival in a density dependent manner, it is possible that they can regulate host numbers. Mathematical models have been used to show that *T. tenuis* could generate red grouse population cycles through their density-dependent influence on host reproduction and survival (Dobson & Hudson 1992). In addition an experiment on wild populations has shown that a reduction in parasite numbers reduced the tendency of the population to exhibit cyclic population crashes, leading to the conclusion that parasites are necessary for these cycles (Hudson *et al.* 1998). However the populations continued to oscillate (see Lambin *et al.* 1999). Models of this host-parasite system have been developed which show evidence of cyclic behaviour (Dobson & Hudson 1992), however

individual-based spatial models comparable to those for the spacing behaviour hypothesis have not been made.

1.5 Objectives

I aimed to test whether the parasite *T. tenuis* has developed resistance to the anthelmintic used to treat managed populations of red grouse. Resistance could have significant implications for population dynamics of both species; I test for anthelmintic resistance experimentally in Chapter 2. The spatial distribution of parasite, both within the host and in the environment, is an important factor in parasite transmission and host population dynamics. In Chapter 3 I describe a detailed analysis of the distribution of parasites in a sample of a wild grouse population, as well as a study of the distribution of red grouse caecal faeces containing *T. tenuis* eggs on a large area of moorland in northern England. In Chapter 4 I use a mathematical modelling approach to examine the role of specific parasite-related parameters on cyclic host populations. I then design an individual-based stochastic model that specifically describes the grouse-*T. tenuis* interaction. In chapter 5 I expand this model to consider the effects of spatial distribution and of host territoriality. These models are used to address the question of whether the parasite *T. tenuis* can cause cycles in red grouse population abundance. I conclude by drawing together the findings of the field studies and theoretical modelling in Chapter 6, the general discussion.

CHAPTER 2

Are populations of *Trichostrongylus tenuis* on treated grouse moors developing resistance to anthelmintics?

ABSTRACT

The population densities of red grouse *Lagopus lagopus scoticus* fluctuate cyclically in some areas of Britain, and reducing the burdens of the nematode parasite *Trichostrongylus tenuis* may be one mechanism of dampening these cycles and of reducing grouse mortality. Grouse are treated to reduce *T. tenuis* burdens through the provision of grit coated with the anthelmintic fenbendazole hydrochloride. Low and frequent uptake of the anthelmintic could result in parasitic resistance to fenbendazole, a potentially serious practical and economic problem. An egg hatch assay was used to test the susceptibility to fenbendazole of *T. tenuis* collected from twelve different moors in the north of England. The moors differed in their use of medicated grit in terms of the amount and frequency of grit provisioning. The concentration of anthelmintic required to prevent 50% of *T. tenuis* eggs from hatching (ED50) was calculated and compared to the moor treatment history. Resistance to fenbendazole has not developed on any of the sites where samples of *T. tenuis* were collected. An ED50 value of more than 0.1μg ml^{-1} (the discriminating dose) can be used as an indicator of resistance. The greatest mean ED50 of any moor was 0.074μg ml^{-1}. There was no difference in the susceptibility of *T. tenuis* from the 12 different moors. Medicated grit enhances the economic viability of red grouse shooting and the management of grouse moors by increasing the July grouse density. It is important to prevent or at least delay *T. tenuis* resistance to fenbendazole so that the efficacy of available anthelmintics is maintained and parasite control measures remain effective. Management practices to monitor and prevent resistance emerging are discussed.

2.1 INTRODUCTION

2.1.1 Anthelminitic treatment of grouse

The parasitic nematode *Trichostrongylus tenuis* has a detrimental effect on the body condition and survival of red grouse *Lagopus lagopus scoticus* (for review see Hudson & Dobson 1991). It causes a reduction in food intake (Shaw & Moss 1990) body condition (Wilson & Wilson 1978; Shaw & Moss 1990) and breeding production of hens (Hudson 1986b; Shaw & Moss 1990; Hudson 1992; Hudson *et al.* 1992b; Newborn & Foster 2002). High parasite intensities can result in death (Wilson & Wilson 1978; Hudson 1986a). Parasite-induced reductions in breeding can, in theory, destabilise population numbers and cause host population cycles (Anderson & May 1978; May & Anderson 1979; Dobson & Hudson 1992). Experimental reduction of *T. tenuis* infections have dampened population cycles and prevented extreme population crashes on moors of Northern England (Hudson *et al.* 1998, but see Lambin *et al.* 1999).

Red grouse management aims to maintain a sustainable harvest of birds for shooting; dramatic decreases in the density of red grouse due to *T. tenuis* result in significant loss of income on many estates (Hudson & Dobson 1989). For approximately 15 years, attempts have been made to reduce the *T. tenuis* burdens of red grouse by anthelmintic treatment and consequently increase survival, improve breeding success and thereby reduce severe population crashes (Hudson 1986a, Hudson *et al.* 1992b; Newborn & Foster 2002).

To control *T. tenuis,* the direct treatment of individual wild birds ('catch and treat') reduces parasite burdens immediately. However, it is time-consuming and is of short-term benefit since treated birds are susceptible to reinfection straight away. Indirect continual treatment has therefore been developed, which involves the provision on the moor of grit, coated with a layer of fat containing the anthelmintic fenbendazole hydrochloride (trade name - Panacur, Hoescht Animal Health, Milton Keynes, UK). Grouse regularly ingest grit to aid physical digestion of heather, and in the process acquire a dose of the anthelmintic. The recommended approach is to place grit piles (0.5-1.0kg) in a lattice pattern over the moor, about 250m apart, providing at least one

pile in each grouse territory (Hudson & Newborn 1995). Fenbendazole is a broad spectrum anthelmintic effective against eggs, larvae and adult *T. tenuis*. It is not soluble, does not break down in daylight and is safe to wildlife at concentrations far greater than the recommended dose (see Hudson & Dobson 1989). Grit has been shown to reduce *T. tenuis* burdens in wild red grouse by up to 44% by 5 months after treatment compared to untreated areas (Hudson 1992; Newborn & Foster 2002). It also increases chick survival by 38% compared to areas with plain grit and therefore enhances the July grouse density (Newborn & Foster 2002).

2.1.2 Anthelmintic resistance

One possible consequence of the use of medicated grit is that a low and frequent uptake of the anthelmintic could provide strong selective pressure driving parasite resistance to fenbendazole. Resistance is present when the frequency of individuals in a population that can tolerate doses of a compound is greater than in a normal population of the same species (Prichard *et al.* 1980). Resistance is heritable (Prichard *et al.* 1980); as selection continues the proportion of resistance genes and therefore the proportion of resistant parasites increase.

There are several reasons why *T. tenuis* might be expected to develop resistance. Grit provides a low but frequent dose of anthelmintic (Hudson 1992). Frequent treatment promotes resistance (e.g. Barton 1983) because it provides frequent opportunities for selection and if drug efficacy is high only worms carrying resistant alleles survive and reproduce (Sangster & Dobson 2002). Also modelling of worm populations suggests that persistent drugs (such as fenbendazole) select more strongly for resistance than short-acting drugs (Dobson *et al.* 1996). In addition biological aspects of the host-parasite system could also contribute. Resistance is most common in direct life-cycle parasites with short generation times (such as *T. tenuis*) because offspring of resistant worms need only infect and reproduce in a new host to contribute to the population's resistant genes (Sangster & Dobson 2002). Red grouse have little, if any, immunity against *T. tenuis* (Shaw and Moss 1989b; Hudson 1992) and so selection pressure for resistance is likely to be relatively high because the host immune response selects parasites irrespective of drug resistance (Sangster 2001). Further, any resistance

could develop quickly because *T. tenuis* are ubiquitous (100% of wild adult birds infected in northern England (Hudson 1986a)), intensity of infection is high (Hudson *et al.* 1985) and individual *T. tenuis* have great longevity (Shaw & Moss 1989a).

Parasite resistance to anthelmintics has been recorded in many animal industries (for review see Conder & Campbell 1995; Sangster & Dobson 2002) and its emergence is a serious issue for health, welfare and economic reasons. The cost of resistance to Australian sheep farmers, for example, has been estimated at US $250 million per year (Besier *et al.*1996). Nematode resistance to benzimidazoles, such as fenbendazole, has occurred in many animal species including sheep, goats, cattle and horses in many parts of the world (Sangster 1999). It can develop very quickly, for example resistance to thiabendazole (TBZ) was reported only three years after commercial release (Drudge *et al.* 1964). Resistance to one benzimidazole results in resistance to other drugs within the benzimidazole group (known as side resistance) (Sangster *et al.* 1985).

A number of *in vivo* tests such as the faecal egg count reduction test (FECRT) and *in vitro* techniques, for example the egg hatch assay (EHA) and the larval development assay (LDA) have been developed to detect anthelmintic resistance (for review see Johanson 1989; Taylor *et al.* 2002). The EHA for benzimidazoles tests the ability of TBZ to inhibit embryonation and hatching of fresh nematode eggs.

2.1.3 Aim

In this chapter I aimed to assess whether *T. tenuis* has developed resistance to fenbendazole on moors in northern England, where grouse have been continuously treated with anthelmintic for up to seven years.

2.2 METHODS

2.2.1 General approach

Tests were conducted on parasite eggs from 12 different grouse moors in the Pennines, northern England. The amount of grit used on the moors ranged from 0 to 187 kg km^{-2} $year^{-1}$ and the amount of time since treatment began ranged from 0 to 7 years (Table 1).

T. tenuis eggs were extracted from five caecal samples from each moor and an EHA used to test the susceptibility of the eggs to thiabendazole (TBZ). The principle of an EHA is to incubate eggs in serial concentrations of the test drug for a pre-determined time and to then count the proportion of eggs that hatch. *T. tenuis* eggs were mixed in serial concentrations of TBZ. Fenbendazole is unsuitable to use in this test because of its poor solubility (Lacey & Pritchard 1986). TBZ is a more soluble benzimidazole and is commonly used as test drug in this assay. Its use has been justified because side-resistance occurs within this group of anthelmintics (Martin *et al.* 1985). The original test was described by Le Jambre (1976) and has been well validated to reliably detect nematode resistance to benzimidazoles (Johansen 1989).

Table 2.1 The quantity of medicated grit used on each of 12 study moors, and the duration of treatment up to the summer of 2002.

Moor	Grit use (kg km^{-2} $year^{-1}$)	Years of treatment	Total grit used (kg km^{-2})
1	55	6	330
2	187	7	1309
3	0	0	0
4	88	3	264
5	87	5	435
6	150	7	1050
7	55	6	330
8	125	5	625
9	55	2	110
10	0	0	0
11	0	0	0
12	0	0	0

2.2.2 Collection of caecal faeces

Each moor was visited once between 10th January and 19th February 2002 when at least 10 separate fresh grouse caecal faeces were collected. (At least 10 were collected although not all were used for laboratory tests). When one sample was collected any others within a 10m radius were not collected to maximize the likelihood that each came from a different bird. An area of approximately $1km^2$ was searched for caecal faeces.

Faecal samples were stored in separate airtight containers, which were filled with water to create anaerobic conditions and were kept at 20°C for a maximum of four days. Unembryonated eggs are required for an EHA because sensitivity to thiabendazole (TBZ) decreases as embryonation proceeds (Coles & Simpkin 1977; Coles *et al.* 1992)). This method of anaerobic storage was therefore used because it prevents nematode egg development for up to 7 days without impact on subsequent development or susceptibility to TBZ in trichostrongyles (Hunt & Taylor 1989).

2.2.3 Extraction of parasite eggs

T. tenuis eggs were recovered from each individual caecal sample by sieving and centrifuging in saturated salt solution as follows: Three sieves (500μm, 63μm and 38μm) were stacked in descending mesh size order. A caecal sample was placed in the top sieve and water washed through the sieves for several minutes until only coarse particles remained in the two sieves on top. The particles remaining on the finest sieve (including eggs) were pipetted in water to a centrifuge tube.

The eggs were centrifuged for 2 minutes at 1000rpm. The supernatant was removed and the filtrate resuspended in 10ml of saturated salt solution. The solution was carefully mixed by inverting the tube 2 to 3 times and was then centrifuged as before. The eggs (which are less dense than the salt solution) were pipetted from the top of the solution into another centrifuge tube. The eggs were diluted in 10ml of distilled water, mixed and centrifuged as before. The supernatant was removed leaving the filtrate (containing eggs) and a small amount of water. The clean sample of eggs was then diluted with distilled water to the required concentration of 1000 eggs ml^{-1}. Samples that did not

contain sufficient numbers of eggs for the EHA (at least 1000 eggs ml^{-1}) or contained eggs that were embryonated were discarded. On six of the moors many caecal samples did not contain enough eggs to carry out the assay and therefore four samples had to be used, on one moor only three samples were suitable. A total of 52 caecal samples were tested from the 12 moors.

2.2.4 Egg hatch assay

The EHA was conducted according to guidelines from the Parasitology division of Moredun Research Institute, Edinburgh. Assays were carried out using Sterilin 24 multiwell plates (BDH, Dorset, UK). Approximately 100 eggs per well were mixed in serial concentrations of TBZ as described below. A replicate assay was done for each test.

A stock solution of TBZ was made by dissolving 0.1g of pure TBZ (Sigma Aldrich, Dorset, UK) in 40ml of dimethyl sulphoxide (DMSO) (BDH, Dorset, UK) followed by 60ml of distilled water. Working concentrations of TBZ (10, 20, 60 and 100 $\mu g\ ml^{-1}$) were made from the stock solution by diluting further with distilled water. 100µl of water containing approximately 100 eggs was pipetted into one side of a well. 10µl of working concentration of TBZ was pipetted into the opposite side of the well to prevent mixing. 1890µl of distilled water was then added and mixed thoroughly giving a total volume of 2000µl. The final concentrations of TBZ were 0.05, 0.1, 0.3 and 0.5$\mu g\ ml^{-1}$ with a control of DMSO solution. Pilot assays showed that egg hatching was unaffected by the concentration of DMSO in the control well and that the range of concentrations of TBZ would allow between 0 and 100% of eggs to hatch.

The plates were incubated in a relative humidity container (to lessen evaporative loss) at 24°C for 48 hours. Following incubation 50µl of lugol's iodine was added to all wells to stop any further parasite development. The number of eggs and first stage larvae at each concentration were counted using an inverted microscope. Egg counts were performed blind with regard to moor and treatment.

2.2.5 Data analysis

Dose-response describes the effect of a range of doses of anthelmintic on parasite egg hatching. Plotting response as probits against log dose generally results in a straight line. From this the concentration of anthelmintic that prevents 50% of eggs from hatching (ED50) in each caecal sample can be calculated (Hewlett & Placket 1979). ED50 – effective dose, is also commonly referred to as LD50 – lethal dose, or EC50 – effective concentration (Sangster & Dobson 2002).

The mean number of eggs and larvae at each drug concentration (from 2 replicates) was calculated. Probit analysis program (version 1.5, U.S. Environmental Protection Agency 1994) was used to calculate the proportion of eggs that failed to hatch (corrected for natural mortality using data from controls) and to carry out probit transformation to calculate the ED50 of each caecal sample. (For some samples ED50 was determined although confidence intervals could not be calculated because the probit model did not fit the data).

I used univariate GLM to test for differences in ED50 among moors. Separate linear regression analyses were used to test for a relationship between the mean ED50 of nematodes from each moor and the duration of grit medication, quantity of grit used, and total grit used (quantity x duration) on each moor. I used t-tests to compare the mean ED50 of treated and untreated moors. Residuals from all analyses were tested for normality. All mean ED50 are stated ± standard errors.

2.3 RESULTS

Figure 2.1 illustrates the ED50 of *T. tenuis* from each individual faecal sample. There was no significant difference in mean ED50 among moors ($F_{11,51}$=1.038, p=0.433) and the variance of ED50 between moors was equal (Levene statistic$_{11,51}$=1.167, p=0.339). The lowest mean ED50 (0.042±0.011μg ml^{-1} TBZ) was on a moor with an intermediate level of treatment (435kg km^{-2} of grit for 5 years), the greatest mean ED50 recorded (0.074 ±0.012μg ml^{-1} TBZ) was on a moor that used 55kg km^{-2} of grit for 6 years, (highest treatment on any moor was 187kg km^{-2} for 7 years).

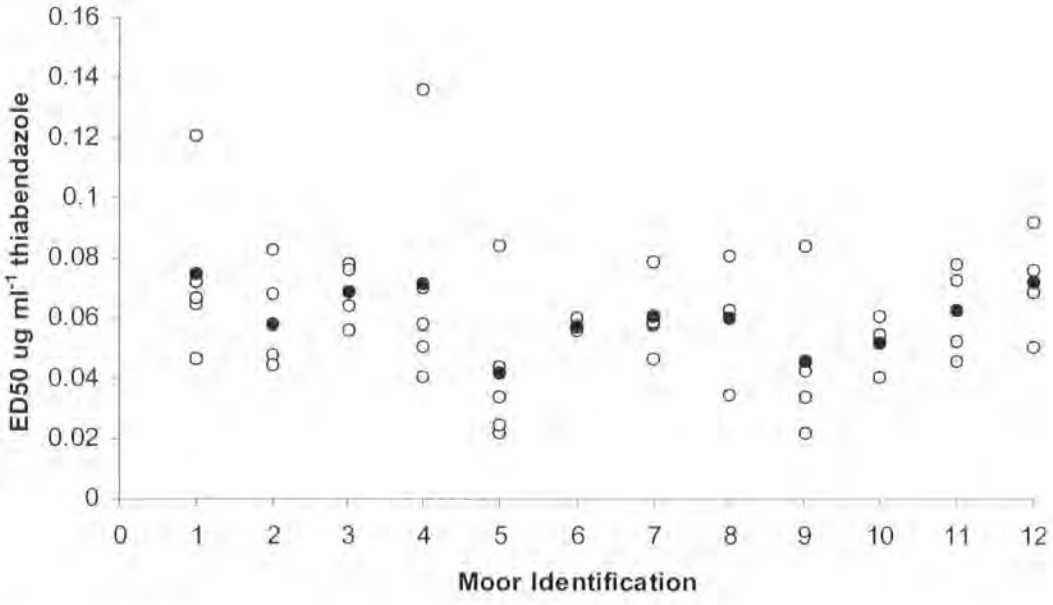


Figure 2.1 ED50 of *T. tenuis* from faecal samples from 12 different moors in the north of England 2002. White circle: ED50 of *T. tenuis* from each caecal sample; black circle: Moor mean ED50. Moor identification details are shown in Table 2.1

The EHA is only sensitive enough to detect resistance if the proportion of resistant worms in the population is more than 25% (Martin *et al.* 1989). An ED50 value greater than 0.1μg ml^{-1} TBZ is often used as an indication of benzimidazole resistance (Coles *et al.* 1992). This discriminating dose can be used for qualitative interpretation of results (Taylor *et al.*2002). Looking for survivors above this value increases the sensitivity of these tests over the calculation of ED50 (Sangster 2001). Although the mean ED50 of

all moors was well below this discriminating dose there were two individual caecal samples with an ED50 greater than 0.1μg ml^{-1} (Figure 2.1). The ED50 values for these samples were 0.121μg ml^{-1} (moor 1 – grit use of 55kg km^{-2} for 6 years) and 0.136μg ml^{-1} (moor 4 – grit use of 88kg km^{-2} for 3 years).

I predicted that ED50 would increase with the length of time a moor had been treated and the amount of grit applied. However there was no significant relationship between the mean ED50 of each moor and the amount of grit used per year ($F_{1,10}$=0.572, p=0.467; Figure 2.2), the number of years grit had been used ($F_{1,10}$=0.184, p=0.677; Figure 2.3), or the total amount of grit used since treatment began ($F_{1,10}$=0.345, p=0.570; Figure 2.4). Altogether 12 moors were studied and a post-hoc power analysis determined that there was sufficient power (0.8) to detect a correlation with r=0.6, however see Colegrave & Ruxton (2003).

Treated moors had a mean ED50 of 0.059μg ml^{-1} (±0.004) (n=8) compared with untreated moors where the mean ED50 was 0.064μg ml^{-1} (±0.003) (n=4). Test of homogeneity indicated that the variance in treated and untreated moors was equal (Levene statistic=0.156, df=1,10, p=0.701) and a t-test indicated no significant difference in the mean ED50 of treated and untreated moors (t=0.815, df=10, p=0.434).

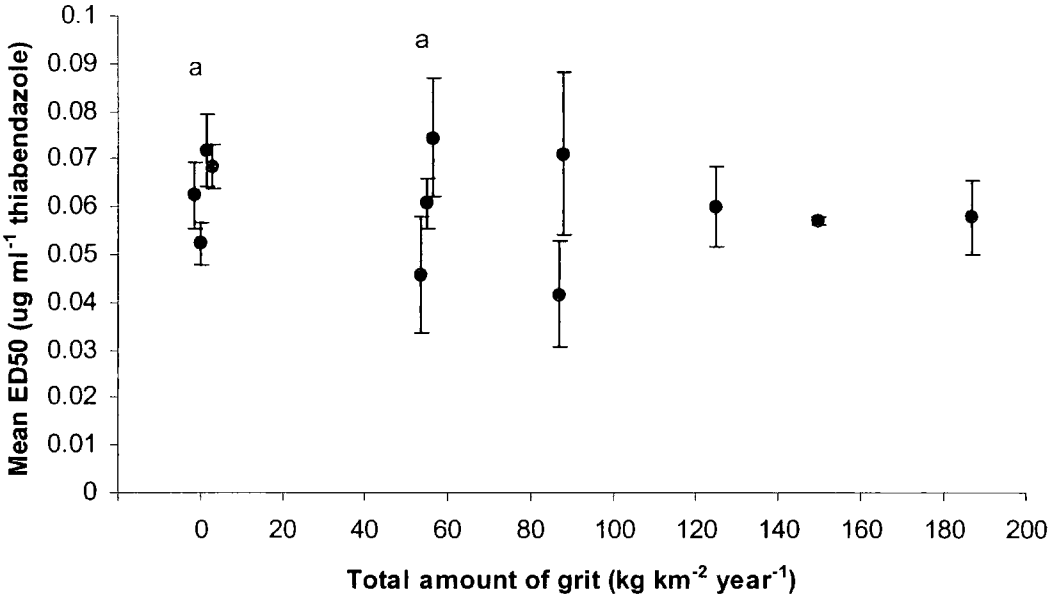


Figure 2.2. Comparison of the mean ED50 of *T. tenuis* from each of 12 moors in northern England in 2002 and the amount of medicated grit used km^{-2} year^{-1}. (a: Points with the same x axis value have been separated slightly).

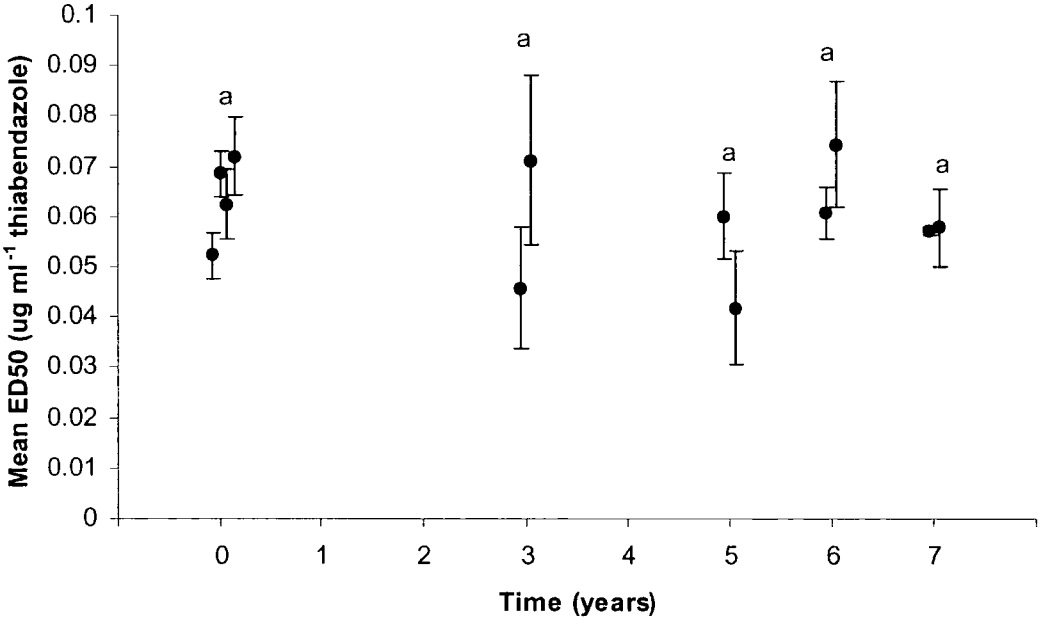


Figure 2.3. Comparison of the mean ED50 of *T. tenuis* from each of 12 moors in northern England in 2002 and the number of years since medicated grit use began. (a: Points with the same x axis value have been separated slightly).

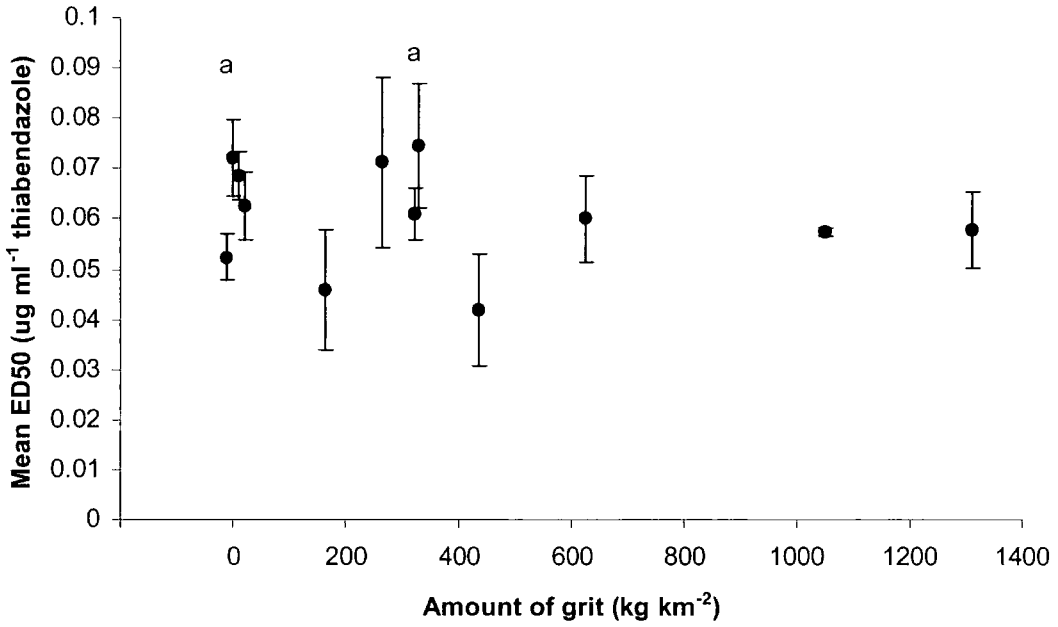


Figure 2.4. Comparison of the mean ED50 of *T. tenuis* from each of 12 moors in northern England in 2002 and the total amount of medicated grit used since treatment began (kg km^{-2} x no. years of use). (a: Points with the same x axis value have been separated slightly).

2.4 DISCUSSION

2.4.1 Detection of resistance

There was no difference in the susceptibility to fenbendazole of *T. tenuis* across 12 different moors with different treatment histories. Overall the mean ED50 on all moors tested was below the discriminating dose, indicating that resistance has not developed on any of the areas under the treatment regimes that have been used. Further, there was no relationship between ED50 and treatment history. Although the mean ED50 of all moors was below the discriminating dose (0.1μg ml^{-1}) that can be used as an indication of resistance (Taylor *et al.* 2002), there were two caecal samples with ED50s exceeding this level. Both were on moors with intermediate levels of treatment.

There are few sensitive methods for measuring resistance and therefore estimates of prevalence are usually underestimates (Sangster 2001). The EHA only detects resistance if the proportion of resistant worms in the population is more than 25% (Martin *et al.* 1989). Measuring resistance at a very low frequency requires sensitive genetic tests (e.g. based on polymerase chain reaction (PCR) assays) although none are used routinely for resistance management (Sangster 2001). PCR can detect 1% resistant individuals in a sample of a population and can also be applied to individual eggs, larvae or adult worms (Roos *et al.* 1995). PCR has been used to identify benzimidazole resistance in some parasites such as the sheep parasites *Haemonchus contortus* and *Trichostrongylus colubriformis* (Roos *et al.* 1995) and the small ruminant nematode *Teladorsagia circumcincta* (Elard *et al.* 1999). Current research aims to test whether the same gene mutation that confers resistance to fenbendazole in these parasites has arisen in *T. tenuis* (L. Keller pers comm.).

2.4.2 Selection for resistance

At the present time there is no evidence of resistance in *T. tenuis* in grouse in the UK. Why this is so and whether resistance could potentially develop is determined by the extent that survivors of drug treatment contribute their genes to future generations (Barnes *et al.*1995). This contribution is determined by factors concerning the drug, the

parasite and the host-parasite system, including frequency and timing of treatment, the life cycle of the parasite and the transmission of parasite to host. There are many reasons why we might expect resistance to develop in this system and therefore we briefly speculate why resistance was not detected on the study areas.

Environmental conditions may be one factor influencing the absence of resistance. For instance, selection for resistance will be slow under environmental conditions that support a high proportion of the parasite population in refugia (i.e. parasites not in contact with the drug such as free-living larvae on the moor) (e.g. Martin *et al.*1981). Moors that are generally wet will support *T. tenuis* refugia and selection for resistance could be relatively weak in these areas. Environmental effects are not easy to predict, for example, Avermectin resistance in cattle parasites has been reported on moist pastures because of the combination of frequent treatment, efficient parasite transmission, short life cycle and poor host immunity (Sangster 2001). One explanation for the absence of resistance in the populations studied is that it has not had time to develop; in which case, there is still the potential for resistance to develop in the future, as it has in the nematodes of domestic livestock (for review see Sangster 1999). It may also be that the grit is used infrequently enough to prevent resistance, because there is a period of approximately 6 months each year where it is not used (Hudson & Newborn 1995), and a frequent turnover of grouse (the approximate life span of a grouse is two years (Jenkins *et al.*1963; Hudson 1986a)).

2.4.3 Controlling the potential development of resistance

Should resistance develop in *T. tenuis* in grouse, it would have severe economic consequences for the estates that rely on grouse shooting for income according to the findings of Newborn & Foster (2002). The development of a new class of anthelmintic drug in the near future seems unlikely (Thompson 1999) and so it is especially important that the efficacy of currently available compounds is maintained. It is therefore worth considering strategies to avoid resistance developing. A number of measures have been recommended to prevent or delay resistance to anthelmintics in

domestic livestock (e.g. Raether 1988; Sangster 1999; Sangster 2001), some of which are applicable to grouse management.

The most successful method to prevent resistance is to reduce treatment frequency (Sangster 1999) even though such a strategy may not achieve the production benefits required (Smith 1990). An alternative strategy is to use doses that will kill parasites completely to prevent any resistant individuals surviving. However controlling the concentration and frequency of dosing are practically impossible when using medicated grit. Increasing the dose may also have the reverse effect of increasing the speed of resistance development by increasing selection on resistant alleles (e.g. experimental evidence (Sangster & Bjørn 1995) and simulations (Barnes *et al.*1995).

Development of resistance can be slowed by rotation of anthelmintics of different groups (Waller *et al.* 1985), although modelling suggests that simultaneous administration may be more effective (Smith 1990) since worms must be resistant to both drugs to survive (Sangster & Dobson 2002). Combination of medicated grit with the 'catch and treat' method using anthelmintics such as Nilverm may therefore be beneficial to red grouse parasite control. Direct dosing would be most effective when *T. tenuis* infection is expected (often in spring (Hudson 1992)) while medicated grit could keep worm burdens at a low level (Hudson 1992).

Integrating chemical and non-chemical control in red grouse management has been considered. Timing of treatment and weather conditions could play a role (Raether 1988) since the free-living larvae and eggs of *T. tenuis* are susceptible to dry conditions (Shaw *et al.* 1989). Geographic information systems have been used to assess infection risk for some parasites (Malone *et al.* 1998; Sutherst 1998) and enhancements of this technique may be useful for predicting infectivity on grouse habitat. Reducing the humidity of the moors by drainage has been suggested as a control strategy (Hudson & Rands 1988), however the efficacy is limited for some moor types such as blanket bog (Hudson & Newborn 1987) and is likely to have deleterious consequences for many species. Finally, alternatives to anthelmintics such as biological control agents,

e.g. fungi (Williams 1997), require further investigation for red grouse management (Hudson 1986a)

2.4.4 Conclusion

Red grouse management relies on anthelmintic drugs for the control of the parasite *T. tenuis*, which has a significant effect on red grouse at the individual and the population level. Although there was no evidence of resistance developing in areas that were tested (despite a range of medicated grit treatments) it is possible that resistance may develop in the future. Parasite resistance to anthelmintics has become a serious economic problem in many animal industries. Methods to prevent the development of resistance in *T. tenuis* have been suggested and responsible efforts by all gamekeepers are required to maintain the efficacy of available compounds so that parasite control measures remain effective.

CHAPTER 3

The distribution of *Trichostrongylus tenuis* eggs in red grouse caecal faeces on Eggleston moor, Teesdale.

ABSTRACT

The spatial distribution of parasites in the environment, and the degree to which they coincide with the spatial arrangement of the hosts, will have an important influence on the host-parasite interaction. Detailed studies on the distribution of *T. tenuis* on an area of moorland in northen England supported the hypothesis that the parasite population is not uniformly distributed among the host population. All grouse sampled were infected with the parasite. Parasite burdens were aggregated among hosts as were parasite eggs in caecal faeces from these birds. Grouse infection was correlated with age and with location of the bird on the moor. In addition I investigated the spatial distribution of *T. tenuis* eggs in caecal faeces across the same moor. Egg concentration was also aggregated among these caecal faeces but was not related to red grouse density. Furthermore *T. tenuis* egg concentration between caecal faeces was only weakly spatially autocorrelated suggesting that additional factors not measured influenced parasite burden.

3.1 INTRODUCTION

3.1.1 *T. tenuis* transmission

The grouse nematode *T. tenuis* is transmitted between adult grouse via the ingestion of infective larvae. *T. tenuis* adults reproduce within the grouse and parasite eggs are deposited on the moor in the grouse caecal faeces, which are usually produced once per day (Moss *et al.* 1993b). Caecal faeces can contain thousands of eggs since each adult female parasite can produce around 100 to 150 eggs per day (Shaw & Moss 1989a; Hudson 1992). Development of eggs to infective third stage larvae depends on humidity (Shaw *et al.* 1989; Watson 1988) and temperature (Shaw *et al.* 1989; Connan & Wise 1993; 1994). Development can take 9 days in optimum conditions (Shaw *et al.* 1989) although eggs can remain viable for several months in late winter (Connan & Wise 1993). Infective larvae migrate from the caecal faeces to the tips of young heather plants (McGladdery 1984; Watson & Hudson 1987; Saunders *et al.* 1999), where they are eaten by grouse (Hudson 1986a; Hudson 1992). Transmission is dependent on a variety factors influencing development, survival and distribution of the parasite eggs, larvae and grouse.

3.1.2 Spatial distribution of parasites

The spatial distribution of parasites in the environment, and the degree to which they coincide with the spatial arrangement of the hosts, will have an important influence on the host-parasite interaction (e.g. Keymer & Anderson 1979). Distribution of larvae will determine the likelihood of parasite ingestion. Ingestion rate will in turn influence adult parasite infection in the grouse population. Of course, the spatial distribution of the parasite and host are strongly interdependent, but many other factors, including environmental variables can also influence the relationship. Ultimately the distribution of parasites among hosts influences host population dynamics and can have a stabilizing or destabilizing influence (Anderson & May 1978; Dobson & Hudson 1992). Despite the importance of this interaction in understanding host-parasite dynamics, little is currently known about the distribution of parasite infective stages in the wild, or how this affects host infection (Shaw & Dobson 1995).

Understanding the relationship between the spatial arrangement of grouse and parasites requires accurate measurement of both populations. This is particularly problematic in the case of parasites, which in their infective stage are small mobile larvae, which can be difficult to detect (*T. tenuis* larvae are approximately 100μm in length). Previous attempts have been made to estimate the availability of *T. tenuis* larvae on vegetation (Hudson 1986a; Shaw *et al.* 1989; Saunders *et al.* 1999), but these studies rarely demonstrated the presence of larvae. Low larval recovery was not due to larvae burrowing inside heather leaflets or accumulating in dew droplets on heather, which fall from the vegetation during sampling (Saunders *et al.* 1999), although it may partially be accounted for by temporal variation, with larvae ascending the vegetation in daylight (Saunders *et al.* 2000). However Saunders *et al.* (2000) suggested that poor larval recovery might be due to a highly aggregated distribution, with restricted hot spots on the moor containing large numbers of infective larvae. This further emphasizes the need for work on the natural spatial distribution of the parasite.

An alternative method of measuring free-living parasite distribution, sampling caecal faeces for egg abundance, may provide a more realistic method of mapping parasite density. Abundance of parasite eggs is likely to reflect the local availability of infective larvae on vegetation. Although larvae do migrate horizontally (as well as vertically), and may travel further than the 10cm recorded by Saunders (1999), lengthy migration is unlikely since this would deplete lipid reserves to levels insufficient to enable successful infection of a new host (Watson 1988). This method of estimating parasite abundance is supported by studies on other trichostrongyle species, which have shown that numbers of larvae on herbage are directly related to numbers of eggs in faecal material (Stromberg 1997).

As well as being correlated with the local abundance of infective stages, the caecal faeces egg concentration might also be used to predict host parasite burdens. This then allows examination of the relationship between host infection and host density. However, egg counts may be an unreliable measure of adult parasite infection if egg production is influenced, for example, by high adult parasite burdens (Moss *et al.* 1993b; Hudson & Dobson 1997; Seivwright *et al.* 2004) or seasonal variation in worm egg production (Moss *et al.* 1993b). Nevertheless, where a sufficiently strong

relationship with adult parasite burden exists, using this as a surrogate is preferable to measuring adult infection rate directly, which requires killing the birds. This type of indirect measure can be particularly useful for long-term studies where parasite burdens of identified birds can be estimated repeatedly.

Estimating the distribution of parasites in the host population is of considerable interest in its own right, since the heterogeneity of parasite abundance can have important consequences for host parasite population dynamics (Grenfell *et al.* 1995). A high degree of parasite aggregation among hosts (a small proportion of hosts infected with a high proportion of the parasite population) tends to stabilise the interaction, whereas weak aggregation can have a destabilizing effect (Anderson & May 1978). Theoretically grouse numbers can cycle when *T. tenuis* aggregation is low, as recorded in some red grouse populations (Hudson *et al.* 1992b) although a high degree of aggregation has been recorded in others (Wilson 1983).

Concentration of eggs in caecal faeces evidently depends on the degree of infection in the host that produced the caecal, but the nature of this relationship could be influenced by host density. If infective parasite density, and therefore transmission efficiency, is related to host density then parasites are likely to play a role in regulating the abundance of the host population (Anderson 1991). However if transmission rate varies with a factor unrelated to host density, such as weather, then parasite burdens may not be related to host density and population cycles are unlikely to occur (Moss *et al.* 1993b).

3.1.3 Aims

In this chapter I address the following questions:

1. What is the frequency distribution of adult parasites and caecal eggs in grouse sampled from the moor? How does spatial location influence this distribution?

2. What is the precise relationship between adult parasite burden and egg concentration in caecal faeces? I will use this information both to assess the utility of faecal egg counts as a surrogate measure of adult infection rate, and to further develop an understanding of factors influencing local parasite abundance.

3. What is the spatial distribution on the moor of parasite eggs in caecal faeces, and what factors determine this distribution?

4. Can parasite egg concentration be predicted across the whole moor from intensive point sampling? If so, I will map the predicted parasite egg density across the whole study site.

5. Does grouse density determine the degree of local parasite infection estimated from caecal egg concentration?

3.2 METHODS

3.2.1 Study area

The study area was a managed grouse moor covering approximately 40 square km of Eggleston moor in Teesdale. The moor was dominated by heather, *Calluna vulgaris*, other plants included rush *Juncus spp.*, bracken *Pteridium spp.* and various grass species typical of Pennine grouse moors. Heather was managed by rotational burning (in patches of 20-30m wide and several hundred metres long). I established 14 parallel transects across the moor, running east-west at 500m intervals (Figure 3.1). Transects ranged between approximately 1.5 and 4km in length, giving a total transect length of approximately 36km.

3.2.2 Grouse caecal faeces collection

I collected grouse caecal droppings by walking each transect (with the aid of a compass and global positioning system (GPS)) and collecting every caecal dropping encountered within 1m either side of the central line. The position of each caecal was recorded using a GPS. Samples were stored in individual plastic bags, which were tied to exclude as much air as possible, and kept in a cool bag until returned to the laboratory on the same day as collection. They were stored at 5°C for a maximum of 4 days. These conditions prevented parasite egg development so that caecal egg contents could be quantified (Seivwright *et al.* 2004).

Two surveys for parasites were made in 2002; once in April (April 9th - 17th) and once in July (July 18th- August 8th). Surveys were completed in the minimum number of days possible, although weather restricted the timing of collection. Surveys were carried out in these months for the following reasons. Caecal was not collected before April to avoid any sudden rises in worm burdens that sometimes occur in March (Moss *et al.* 1993b). It was necessary to avoid the time when hens were incubating (from late April until mid May) because they stop producing caecal (Hudson & Dobson 1989; Hudson *et al.* 1992a). Peak grouse egg hatching on the moor was approximately May 22nd (P. Warren pers. comm.) and it was not possible to survey the moor when the chicks were very young. It was hoped that the second survey would be in June 2002 however the weather conditions were not favourable for most of the month and access to the moor

was not obtained until July. At this time the chicks were old enough to produce caecal that was indistinguishable from adult caecal. As a result the survey in July included caecal samples from young birds as well as those more than one year old.

3.2.3 Egg concentration in caecal droppings

Egg concentration in caecal droppings was determined using the McMaster egg counting method (MAFF 1978; Sloss *et al.* 1994), which has been used in previous studies to assess egg concentration in grouse caecal faeces (e.g. Shaw 1988; Shaw & Moss 1989a,b; Shaw *et al.* 1989; Hudson & Dobson 1997). Caecal samples collected in July were weighed before egg content was assessed.

Each caecal was mixed thoroughly, and a sample of 1.0g was then mixed with 14ml of saturated salt solution. A sample of this solution was pipetted under the two bridges of a McMaster slide (each chamber contained 0.15ml of solution). Sufficient time was allowed for the parasite eggs to float to the surface (becoming clearly visible beneath the glass bridge under a microscope). The number of eggs in the two separate compartments was counted using a marked grid. The two counts were considered accurate if the standard deviation of the mean was within 25% of the mean (Thienpont *et al.* 1979). If inaccurate, the two counts were repeated with fresh thoroughly mixed solution. The number of eggs per gram of caecal was calculated.

Caecal faeces samples had been present on the moor for different lengths of time before being collected. It is possible that drying over time might affect the egg concentration recorded in the laboratory (drier caecal being expected to contain more eggs g^{-1}). Some of the caecal samples collected in July (n=182) were therefore, analysed for moisture content by drying to constant weight. Samples of 1.0g of caecal were dried in an oven at 60°C for several days. They were then weighed, dried for a further 48 hours and then reweighed. The process of drying and re-weighing continued until two subsequent weights were the same (± 0.01g).

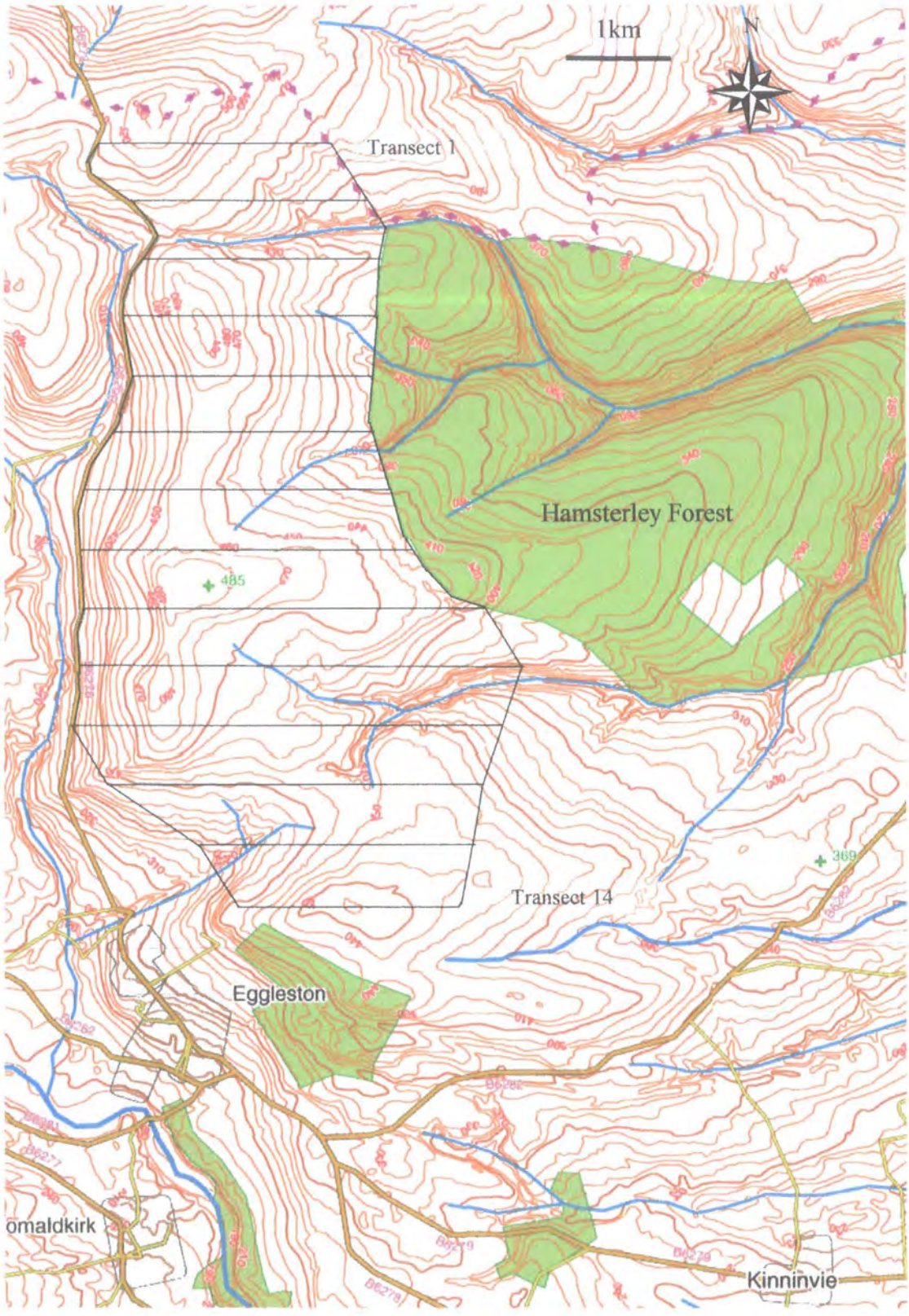


Figure 3.1 Eggleston moor, Teesdale, UK.
Study area is shown with transects running east- west at 500m intervals

3.2.4 Grouse adult parasite burdens

Adult parasite concentration in a sample of red grouse and the parasite egg concentration in the caecal from the guts were estimated from 55 grouse. Grouse sport shooting occurred on Eggleston moor during September; a sample of carcasses was collected for analysis on two shooting days: September 3rd (n=10 young - hatched that year, and 15 adult birds – more than one year old) and September 6th 2002 (n=5 young and 25 adults). The birds were divided by age, into young or adults by gamekeepers at the site of collection. Ages were estimated by inspecting the toenails for scars in old birds and the condition and moult of the primary feathers as described by Hudson (1995). Birds were shot on two different areas of the moor: from north of transect number six, and south of transect number eight (Figure 3.1). I thus further subdivided the birds into groups on this basis, termed north and south. This was appropriate because grouse are sedentary and move only small distances (Jenkins *et al.* 1963). Although shooting causes disturbance to the birds it is very unlikely that those from the north or south would be shot in a different area to their territory (P. Warren pers. comm.).

The intestine including caeca were removed from the dead birds, stored in a cold bag and returned to the laboratory immediately. One caecum of the gut was used straight away to assess parasite egg concentration in caecal faeces, the other was used to assess adult parasite concentration (see below). The caecum used to assess the adult parasites was stored in a freezer at –20°C until required. Freezing does not have a detrimental effect on the subsequent estimation of adult parasite burden (Hudson & Newborn 1995).

Worm eggs were sampled from one caecum by collecting approximately 1g of caecal faeces from the proximal end (the faeces that would be voided next (Hudson & Dobson 1997)). The egg concentration in 1g (+/-0.01g) of this faeces was estimated using the McMaster method described in section 3.2.3.

Adult worm infection was determined from the second caecum using a method described in detail by Wilson (1983), which has often been used to estimate grouse parasite burden (e.g. Shaw & Moss 1989a; Hudson 1986b, Hudson & Dobson 1997). Worms were extracted by cutting the caecum length ways and then into short

(approximately 5cm) sections. The caecum was carefully washed in an 800μm mesh sieve on top of a 200μm sieve so that the adult worms were collected in the lower sieve. Contents of the lower sieve were washed into a beaker and diluted to 300ml of water and mixed thoroughly. The number of worms in three subsamples of 10ml were counted in a petri dish placed on a dark background. Some of the worms were broken and therefore all full worms and any that were more than half the length of the average worm were counted (Hudson & Newborn 1995). The number of worms per bird was calculated by adding the three counts in 10ml, multiplying up to 300ml (total volume from one caecum) and multiplying by two because birds have two caecae (I assumed that there was an equal number of worms per caecum (Wilson 1983; Hudson *et al.* 1992b)).

3.2.5 Grouse density

The spring and summer grouse density was assessed on the same moor by other researchers concurrently with the parasite survey. The spring survey was conducted between 22nd and 27th of March 2002 and the summer survey between 14th and 18th of July 2002. In brief, grouse density was estimated by transect counts with the aid of a pointing dog as described by Hudson & Newborn (1995). The same transects were used for both grouse density estimation and collection of grouse caecal faeces (Figure 3.1).

3.2.6 Data analysis

All analyses were done using SPPS (Version 11, SPSS, Inc. Chicago, Illinois) except where stated. All means are stated with standard error. Grouse parasite burdens were classed as aggregated if the variance was greater than the arithmetic mean (Shaw & Dobson 1995). When distributions were aggregated the geometric mean (with standard error) is stated, because the arithmetic mean provides a biased estimate larger than the true average (Hudson & Newborn 1995).

Aggregated spatial patterns in biological populations frequently follow a negative binomial distribution (for review see Shaw & Dobson 1995). (There are, however, many aggregated patterns that are not adequately described by this distribution (Krebs 1989)). For comparison with other host parasite studies (e.g. Anderson & May 1978; Wilson 1983; Dobson & Hudson 1992), the distribution of *T. tenuis* adults among hosts and the

distribution of parasite eggs in caecal samples were compared to the negative binomial distribution (GenStat, version 6.0, VSN International U.K.). For a given mean, the parameter k provides an inverse measure of aggregation of parasites between hosts (e.g. Anderson & May 1978; Dobson & Hudson 1992). Larger values of k indicate a low degree of aggregation and smaller values indicate a high degree of clumping.

I used univariate GLM to test whether age group and location on the moor influenced parasite burden of birds or the egg concentration in caecal faeces produced by those birds. I also tested whether caecal egg count could accurately predict the adult parasite burden (taking into consideration bird age and location on the moor). Finally I tested whether parasite fecundity (number of eggs produced per parasite) varied according to adult parasite burden within the bird. In all tests residuals were tested for normality and the dependent variable log transformed as appropriate.

Since some caecal faeces may have dried more than others on the moor before collection, I used Spearman rank correlation to assess whether water content of caecal faeces affected parasite egg content. Concentration of eggs in caecal faeces was heavily skewed, transformation did not normalise the data. Water content of the caecal faeces (measured as % of total mass) was arcsin square root transformed.

I used a repeated measures GLM to test for changes between April and July in 4 measures of egg abundance: 1) the number of caecal faeces per transect; 2) mean egg concentration per transect; 3) maximum eggs in a caecal per transect; and 4) total eggs per transect. To determine whether changes on transects occurred over shorter distances, transects were also divided into 500m sections and the same tests (including transect section as a factor) were performed. These variables were square root transformed when residuals were non-normal.

The position (and corresponding egg content) of each caecal sample found on the moor was mapped using ArcMap (Version 8.3, ESRI Inc., Redlands, California, USA). I assessed whether parasite density could be predicted across the whole study site by interpolating from the points where caecal faeces were collected. This type of interpolation relies on sampled points being spatially autocorrelated. Positions of strong

similarity (or dissimilarity) are found by comparing each point with every other point. For example, positive autocorrelation would occur if caecal samples close together had similar egg concentrations. Negative spatial autocorrelation would occur if caecal egg concentration at one point was dissimilar to surrounding points. The spatial autocorrelation of caecal egg concentration was tested using the Moran's I statistic (Rook's case add in program for Microsoft Excel; Sawada 1999).

At each point where a grouse caecal faeces was found, the grouse density at that point was recorded from a map of grouse density derived from geostatistical estimates based on field surveys (using the 'sample' command in ArcMap). Grouse density was measured and the maps constructed by P. Warren, Game Conservancy Trust. The relationship between grouse density and parasite egg concentration was then analysed. The frequency of egg concentrations was heavily skewed, and these data could not be normalised. I therefore divided egg concentration into 2 categories of approximately equal size, and analysed the relationship with grouse density using binary regression. In addition I looked more closely at the relationship using a univariate GLM to test the relationship between number of eggs and grouse density (caecal faeces without eggs were removed from the analysis). Again, residuals were tested for normality and the dependent variable log transformed where appropriate.

3.3. RESULTS

3.3.1 Adult *T. tenuis* infection in a sample of the grouse population

Inspection of the shot grouse (40 older than 12 months and 15 less than 6 months old) revealed that all were infected with *T. tenuis*, with a geometric mean worm burden of 641.21 (x/÷1.15) worms bird^{-1}. Parasite burdens were larger in older grouse than in young birds (adult: 913.06 (x/÷1.13) parasites bird^{-1}; juveniles: 250.61 (x/÷1.33); discussed further below). While the majority of young grouse (67%) were infected with fewer than 500 worms, by adulthood this proportion of birds carrying this lower level of infection had dropped to 20%. However even among adult birds, the majority (58%) had fewer than 1000 parasites (Figure 3.2).

Overall the distribution of parasites per host was aggregated (variance greater than the mean), indicating that most grouse had a relatively low parasite burden, with a few grouse carrying the majority of the total parasite population. The data for young and old birds combined did not fit the negative binomial distribution (deviance=9.97, df=4, p=0.041). However, the distribution of worms in the young and old grouse treated separately was not significantly different from the negative binomial distribution (deviance=3.34, df=3, p=0.603 k=1.24, and deviance=0.27, df=1, p=0.342, k=1.81 respectively).

After transformation, the data did not depart significantly from a normal distribution, and I used general linear models to compare infection rates between groups. Parasite burden was significantly influenced by the interaction between bird age and position on the moor ($F_{1,54}$=11.99, p=0.001). This demonstrates that adults carried significantly more parasites than juveniles in the north, while there was little or no age difference in parasite burden in the south of the moor (Figure 3.3).

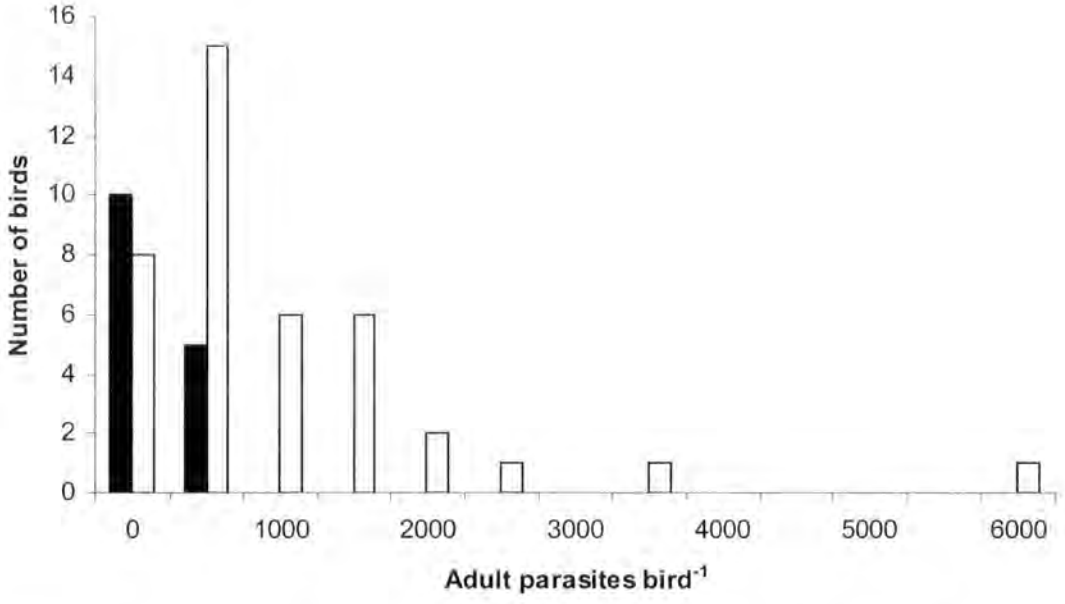


Figure 3.2 Frequency distribution of *T. tenuis* adult parasites in young (black) and adult (white) grouse.

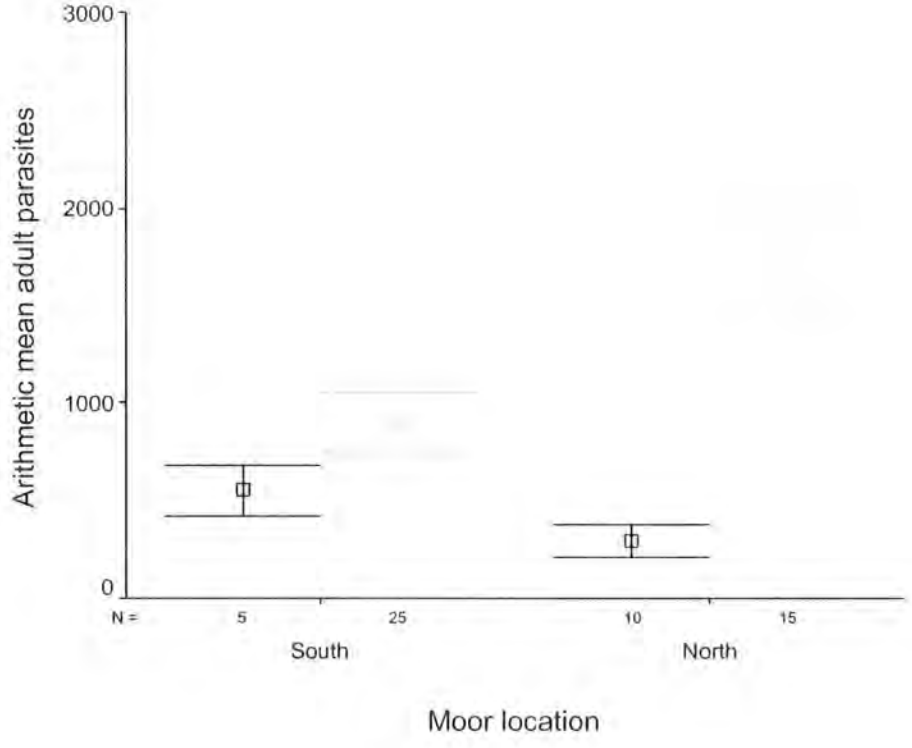


Figure 3.3 Adult parasite burdens in young (black) and adult (grey) grouse on the north and south of Eggleston moor.

3.3.2 The relationship between adult parasite burden and caecal faeces egg concentration

As well as examining the adult parasite burden, I also used this sample of dead grouse to look at the distribution of eggs in caecal faeces taken directly from the birds. All of the 55 caecal faeces sampled contained parasite eggs; with a geometric mean of 15754.08 (x/÷1.27) eggs g^{-1} caecal (young birds: geometric mean=5602.5 x/÷1.50; old birds: geometric mean=23215.18 x/÷1.32). Similarly adult birds faeces contained significantly more parasite eggs than young birds ($F_{1,54}$=7.99, p=0.007). Position on the moor did not significantly affect egg concentration ($F_{1,54}$=0.59, p=0.447). There was no significant interaction between age and position ($F_{1,54}$=0.45; p=0.507). Distribution of parasite eggs among caecal faeces was aggregated, in concordance with the observation for adult parasites in the same sample of grouse. The majority of young grouse (57%) produced caecal containing less that 5000 eggs g^{-1} of caecal faeces, compared to only 17.5% of adult birds. (Overall 27% of caecal faeces contained less than 5000 eggs g^{-1} of caecal faeces).

As predicted, there was a significantly positive relationship between egg concentration in caecal and the corresponding adult parasite burden ($F_{1,54}$=7.80, p=0.007; Figure 3.4). The age of the bird ($F_{1,54}$=0.87, p=0.356) and position on the moor ($F_{1,54}$=0.98, p=0.326) were included as factors in this model; removing these and pooling all birds irrespective of age or position strengthened the relationship between adult worm burden and egg concentration (F_{54}=17.55, p<0.001). Despite this strong relationship, caecal egg concentration explained a relatively small 24.9% of the variance in adult worm burden. While this limits the predictive power of caecal egg concentration in estimating adult parasite burden, the relationship is significantly strong for egg burden to be interpreted as an approximate surrogate for the level of adult infection.

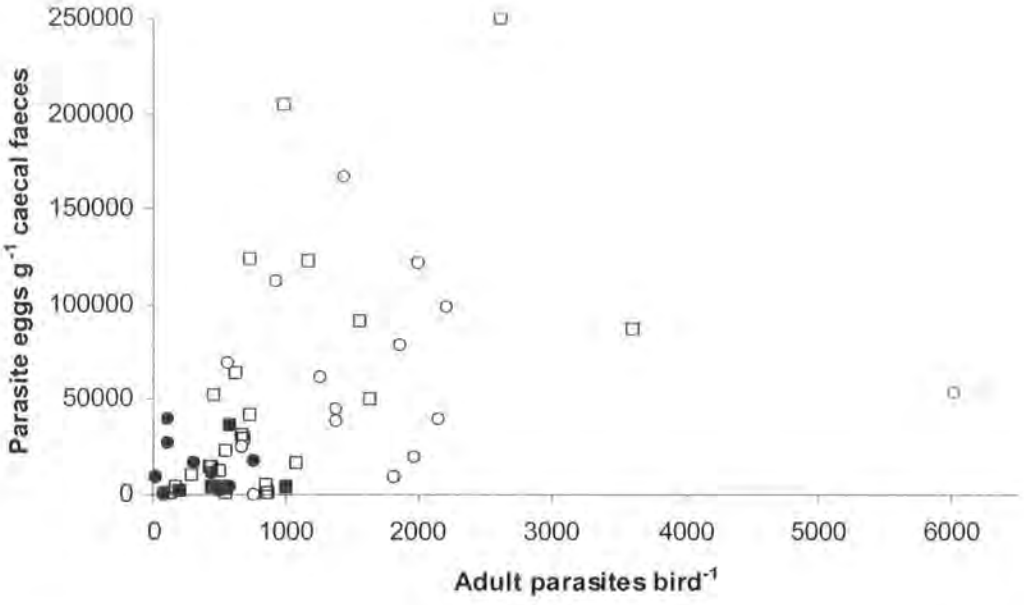


Figure 3.4 Adult worm burdens and concentration of eggs in caecal faeces in all birds sampled from Eggleston moor. Total number of birds = 55. Young birds (black), adult birds (white), birds in the south (squares), birds in the north (circles).

I also tested whether the relationship between egg production and adult parasite burden could be improved by standardizing egg production per adult parasite (mean 61.46±12.41 eggs g^{-1} caecal; Figure 3.5). However individual grouse parasite burden did not significantly affect the number of eggs produced per parasite ($F_{1,54}$=0.90, p=0.765). Egg production per adult parasite was not influenced by the age ($F_{1,54}$=0.45, p=0.506) or position of the bird ($F_{1,54}$=1.15, p=0.289).

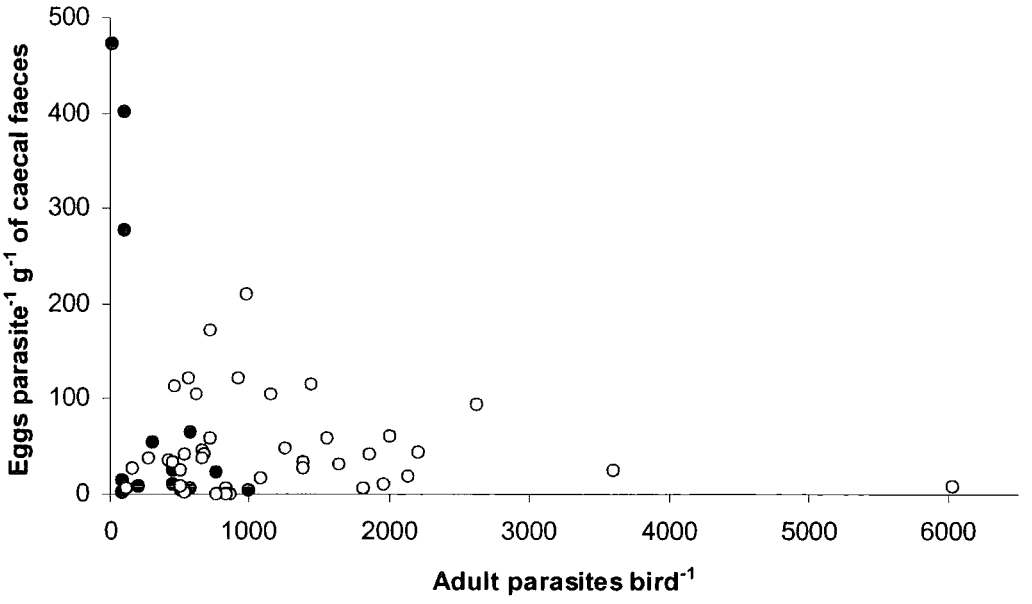


Figure 3.5 Individual adult parasite egg production from a sample of birds from Eggleston moor. Young birds (black), adult birds (white).

3.3.3 Parasites in caecal faeces collected directly from Eggleston moor

Parasite egg concentration

As an additional method of examining the spatial distribution of *T. tenuis*, I collected 387 caecal faeces from 14 transects on Eggleston moor (137 in April and 250 in July 2002). As caecal egg concentration is a relatively weak predictor of adult parasite burden (section 3.3.2), interpretation of individual grouse infection levels based on this measure must be treated with some caution. However caecal egg concentration is also useful in its own right, since it provides information on the availability of infective larval stages that will emerge from these eggs.

I confirmed that the relative desiccation of caecal had no effect on the estimate of egg concentration ($R_{s,\ 181}$=-0.10, p=0.196; Figure 3.6). Variation in egg concentration of caecal faeces was therefore not due to the differing length of time that faeces had been exposed on the moor.

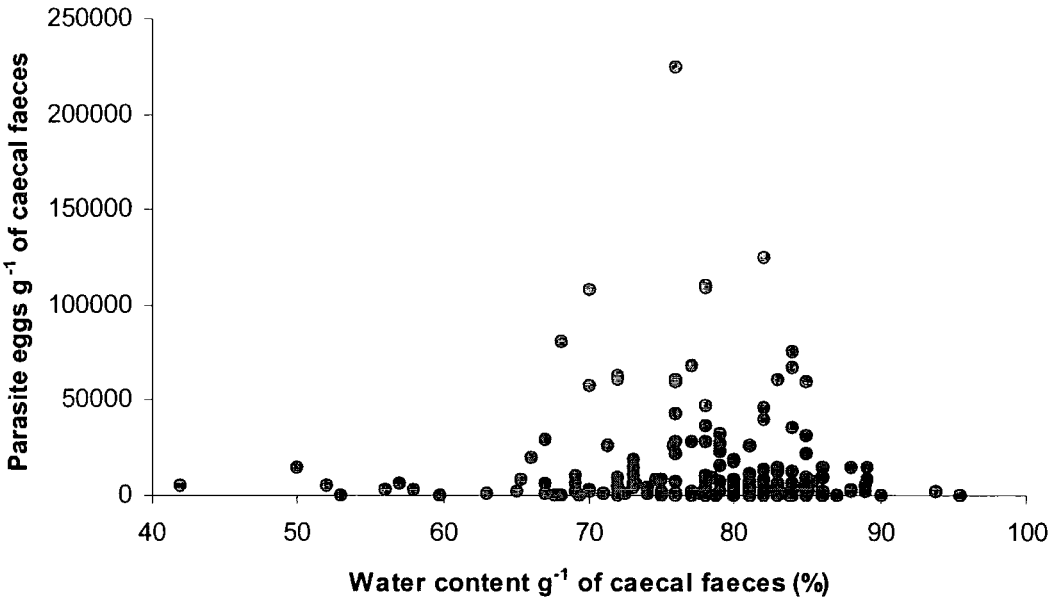


Figure 3.6 Water content and parasite egg concentration of caecal collected on Eggleston moor in July.

In comparison with the caecal collected directly from dead birds (all contained parasite eggs), 99% (136/137) of the caecal faeces contained *T. tenuis* eggs in April compared to 90% (225/ 250) in July (Figure 3.7). In agreement with the data from caecal collected from dead birds, parasite egg concentration in the caecal faeces collected from the moor was also aggregated. Most of the caecal faeces contained relatively few parasite eggs, while a few contained a high proportion of the parasite eggs. In April 32% (44/137) of the caecal contained less than 5000 eggs compared to 56% (140/250) in July. By the following September this percentage had dropped to 27% of caecal faeces containing less than 5000 eggs g^{-1} (in faeces sampled directly from dead birds, section 3.3.2).

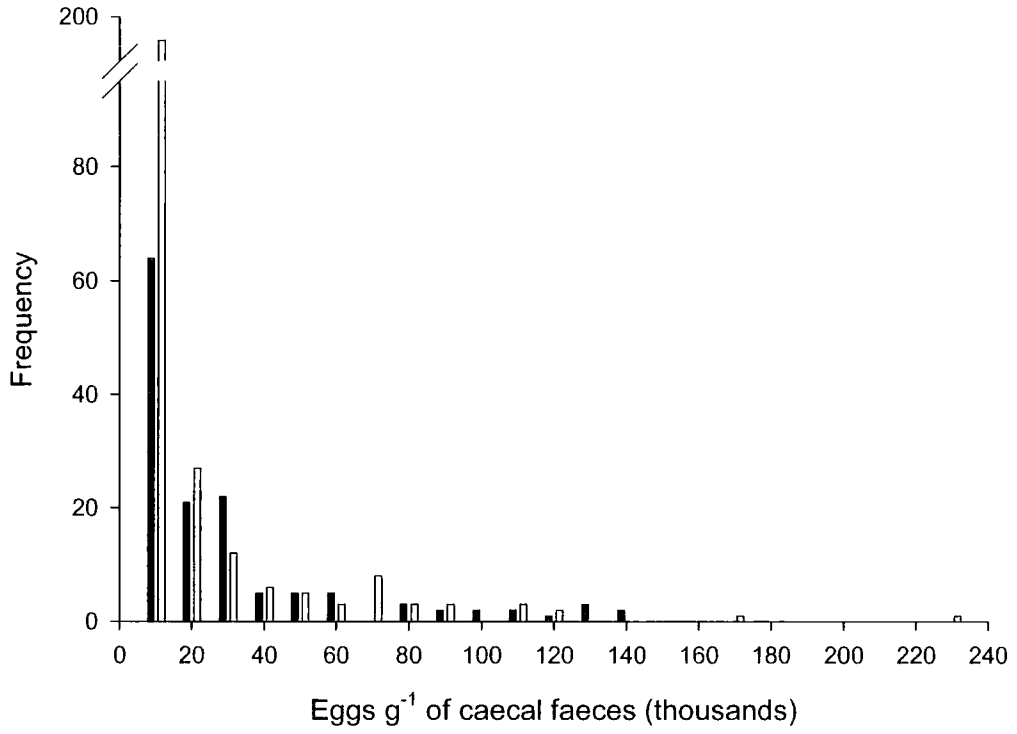


Figure 3.7 Distribution of eggs in caecal samples collected from Eggleston moor in April (black) and July (white).

Was there a change between April and July?

The geometric mean concentration of parasite eggs in April was 9148.26x/÷1.165 eggs g^{-1} of caecal, (range 0 to 138,040 eggs g^{-1} caecal) and in July was 1722.92 x/÷ 1.228 eggs g^{-1} of caecal, (range 0 to 224,500 eggs g^{-1} of caecal).

Since the distribution of parasite eggs among caecal was highly aggregated, the geometric mean provides a more accurate description of the data than the arithmetic mean. The geometric mean cannot be calculated for data that include zeros. Therefore where zero eggs were recorded (n=1 in April; n=25 in July), the zero was replaced with 0.5 for calculation of the geometric mean. The next lowest concentration of eggs the caecal was 46.7 (April) or 50 (July). Adding 0.5 to the zeros is therefore unlikely to influence the accuracy of the geometric mean.

The absence of repeated measurements precludes using these data to draw conclusions about seasonality; nevertheless it is possible to test for short-term temporal stability in parasite burdens. Reproduction and maturation of young birds took place between these two sampling periods, so an increase in caecal production was expected; this prediction was supported ($F_{1,13}$=39.70, p<0.001) (Figure 3.8). However month did not significantly affect the mean concentration of eggs per transect ($F_{1,13}$=0.31, p=0.587), the maximum egg concentration per transect ($F_{1,13}$=0.003, p=0.961) or the total number of eggs on the transect ($F_{1,13}$=0.33, p=0.577).

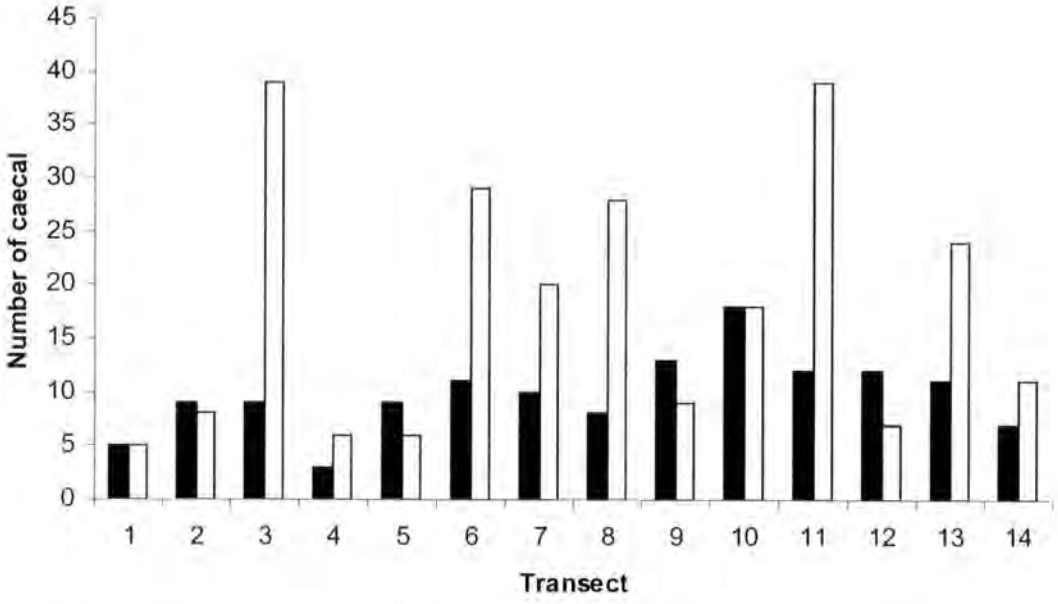


Figure 3.8 Number of caecal collected on each transect in April (black) and July (white).

To determine whether changes on transects occurred over shorter distances transects were divided into sections of 500m. As before, caecal production increased from April to July ($F_{1,48}$=14.04, p<0.001). However the interaction between month and transect approached significance in this model ($F_{13,48}$=1.91, p=0.053), suggesting a greater increase in some transects compared to others (Figure 3.8). After including location in the model of egg production, there was still no significant effect of month on eggs per caecal ($F_{1,48}$=3.17, p=0.081), maximum number of eggs in the section ($F_{1,48}$=0.004, p=0.948) or the total number of eggs in the section ($F_{1,48}$=0.09, p=0.763).

3.3.4 The spatial distribution of *T. tenuis* eggs across the moor

The position and egg concentration of each caecal sample collected on the moor was mapped using ArcGIS (Figure 3.9). I used these data to test the spatial autocorrelation of egg concentration in caecal samples across the study site over different spatial distances. All possible sample pairs are grouped into classes of approximately equal distance (lags). Moran's I measures the correlation between all possible pairs of points at each lag (Figure 3.10). Moran's I has an expected value of near 0 for no spatial autocorrelation, with negative and positive values (between –1 and 1) indicating negative and positive autocorrelation respectively. Moran's I can be thought of as a correlation coefficient where a value greater than approximately 0.7 (or less than –0.7 for negative correlation) would be considered a strong correlation (Fowler *et al.* 1998). In April egg concentration in caecal was positively correlated for lags between approximately 400 and 1000m, suggesting autocorrelation among samples located between 400m and 1000m apart. In July egg concentration in all caecal samples was positively correlated for lags up to at least 1700m. However in both surveys, the value of Moran's I was relatively small (even where Moran's I was significant) indicating weak autocorrelation. Autocorrelation was too weak to allow interpolation of parasite egg distribution across unsampled sections of the moor. Despite the absence of strong autocorrelation noted above, figure 3.9 highlights areas where caecal contain high numbers of eggs, and suggests some degree of spatial clumping in egg burden.

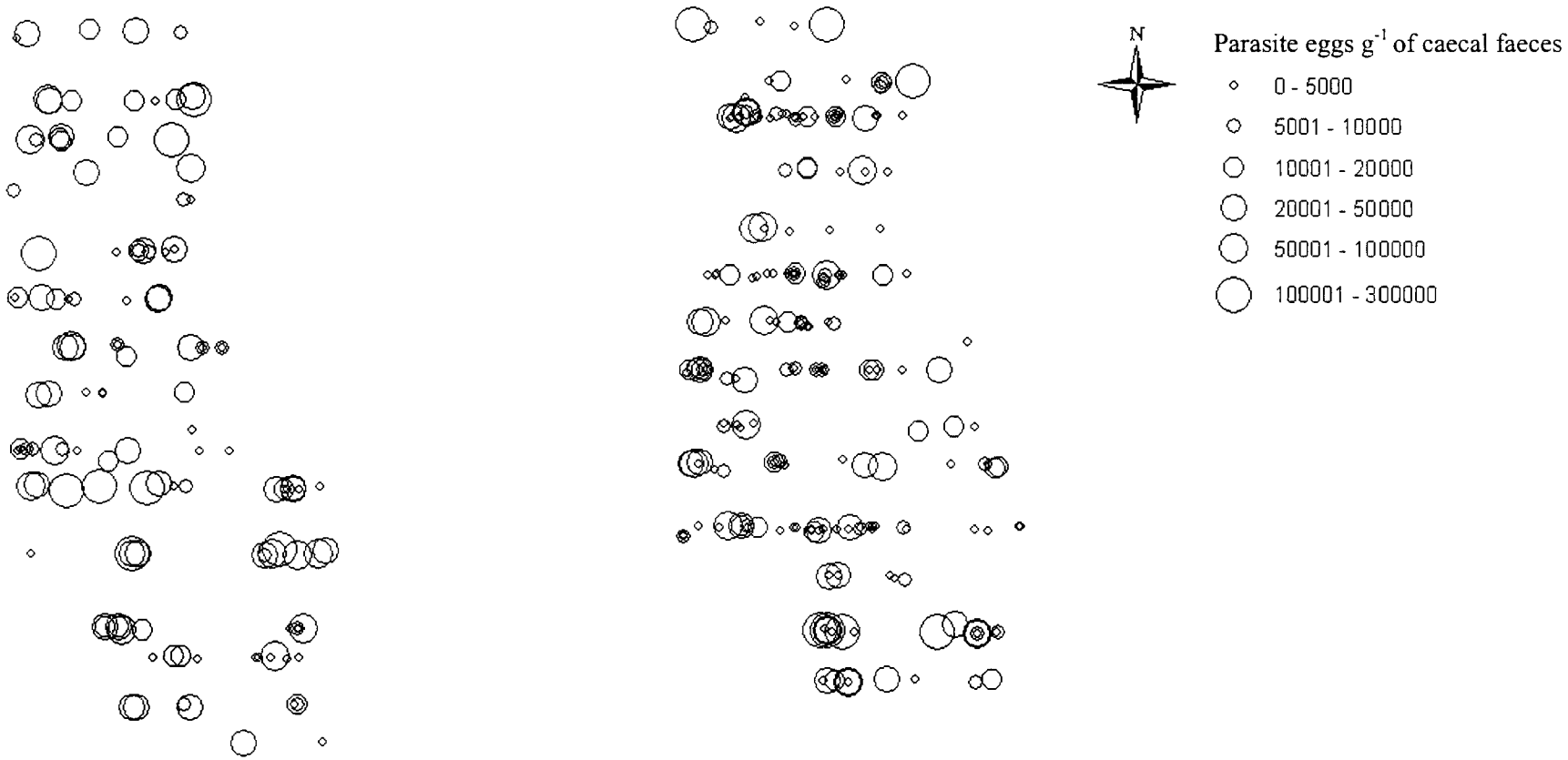
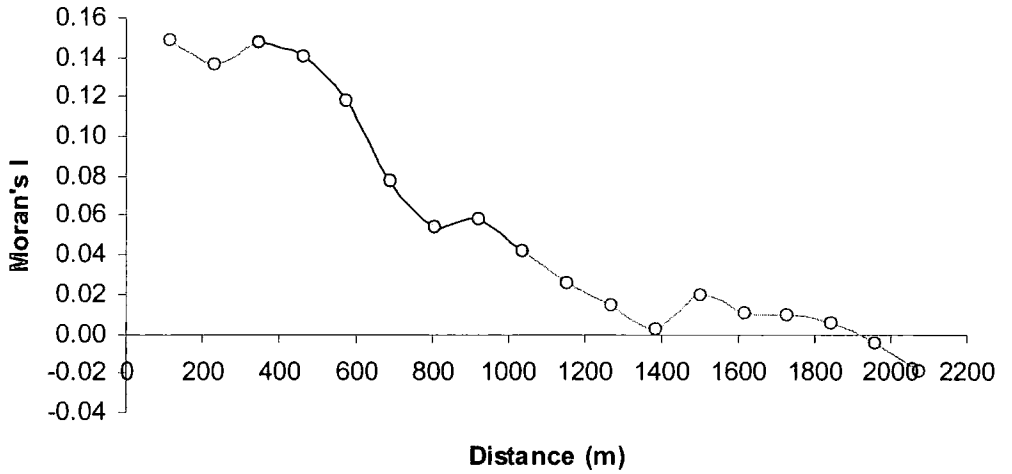


Figure 3.9 Distribution of parasite eggs in caecal faeces on Eggleston moor, Teesdale in April (left) and July (right) 2002.

April



July

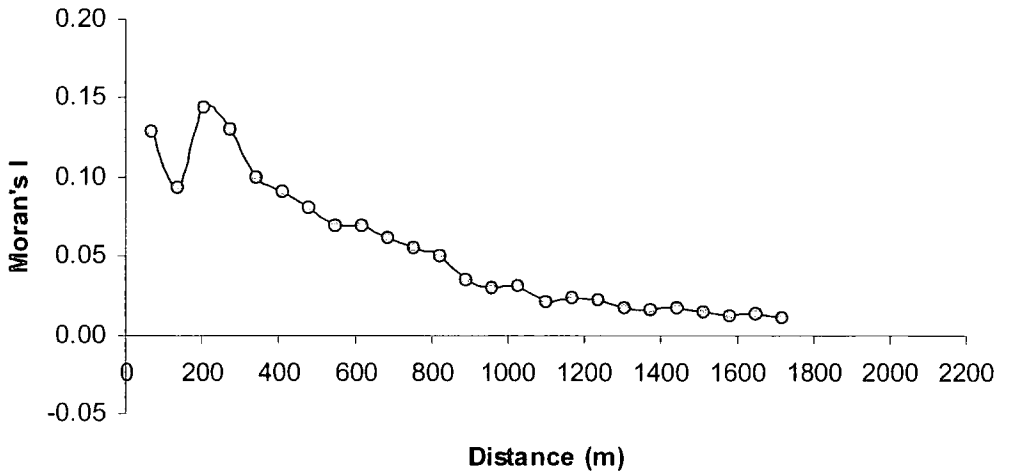


Figure 3.10 Autocorrelation statistic, Moran's I, for egg concentration in caecal faeces on Eggleston moor in April and July. The y axis indicates the value of Moran's I calculated from all possible pairs of sample locations that are separated by that lag distance (x axis). Significant autocorrelation is indicated by black circles, no significant autocorrelation by white circles.

3.3.5 Is concentration of eggs in caecal faeces related to grouse density?

I have demonstrated that the concentration of eggs in caecal faeces was largely independent of spatial location on the moor and the month of sampling. In samples of dead birds it appeared to be related to age of the host. I tested one further possible predictor of caecal egg concentration, namely the local density of the host population.

The grouse density and concentration of parasite eggs in the caecal faeces is illustrated in April (Figure 3.11) and in July (Figure 3.12). For analysis (below) I removed data where egg concentration had been measured but there was no corresponding host density measurement (In April n=132 (5 points were removed; July n=244, 6 points were removed). Inconsistency was due to caecal measurements taken beyond the boundaries used for grouse density measurement.

Because there were large numbers of caecal faeces containing relatively low levels of parasite eggs I compared grouse density with egg concentration divided into two groups of approximately equal numbers in a binary logistic regression. The two groups were low egg concentration (<5000 eggs g^{-1} of faeces; mean 2200 eggs g^{-1}) or high egg concentration (5000-140000 eggs g^{-1}, mean 34600 eggs g^{-1}). Grouse density had no significant effect on egg concentration category (low versus high) including transect as a factor, in April (Wald $statistic_1$=2.44, p=0.118) or July (Wald $statistic_1$=0.47, p=0.495). Since there could be a temporal lag in the relationship between caecal egg concentration and grouse density, I compared caecal egg concentration in July to the grouse density in the previous April in a binary logistic regression. April grouse density was not related to July egg concentration (Wald $statistic_1$=0.00, p=0.999).

I then looked at a more subtle relationship between nonzero egg concentration and grouse density. Caecal faeces in which no eggs were recorded were removed from the analysis, leaving the sample size of 131 in April and 225 in July. A univariate GLM on data collected in April revealed a marginally significant relationship between bird density and parasite egg concentration in caecal faeces ($F_{1,116}$=3.932, p=0.05) (Figure 3.13a). Transect, included as a factor, was also significant in this model ($F_{13,116}$=2.553, p=0.004) as a result of the significant variation in egg concentration across the moor. In July there was no significant relationship between bird density and parasite egg concentration in caecal faeces ($F_{1,204}$=1.31, p=0.255). Transect included as a factor, was not significant in this model ($F_{13,204}$)=1.63, p=0.08) (Figure 3.13b). Again in case of a time lag between grouse density and caecal egg concentration I compared grouse density in April with egg concentration in July using a univariate GLM. The relationship was not significant (Figure 3.13c), ($F_{1,204}$=0.43, p=0.512). Transect, as a fixed factor, was also not significant ($F_{13,204}$=1.62, p=0.083).

Figure 3.11 Egg concentration in caecal faeces compared to grouse density on Eggleston moor in April. Grouse density map reproduced with permission of P. Warren, Game conservancy Trust.

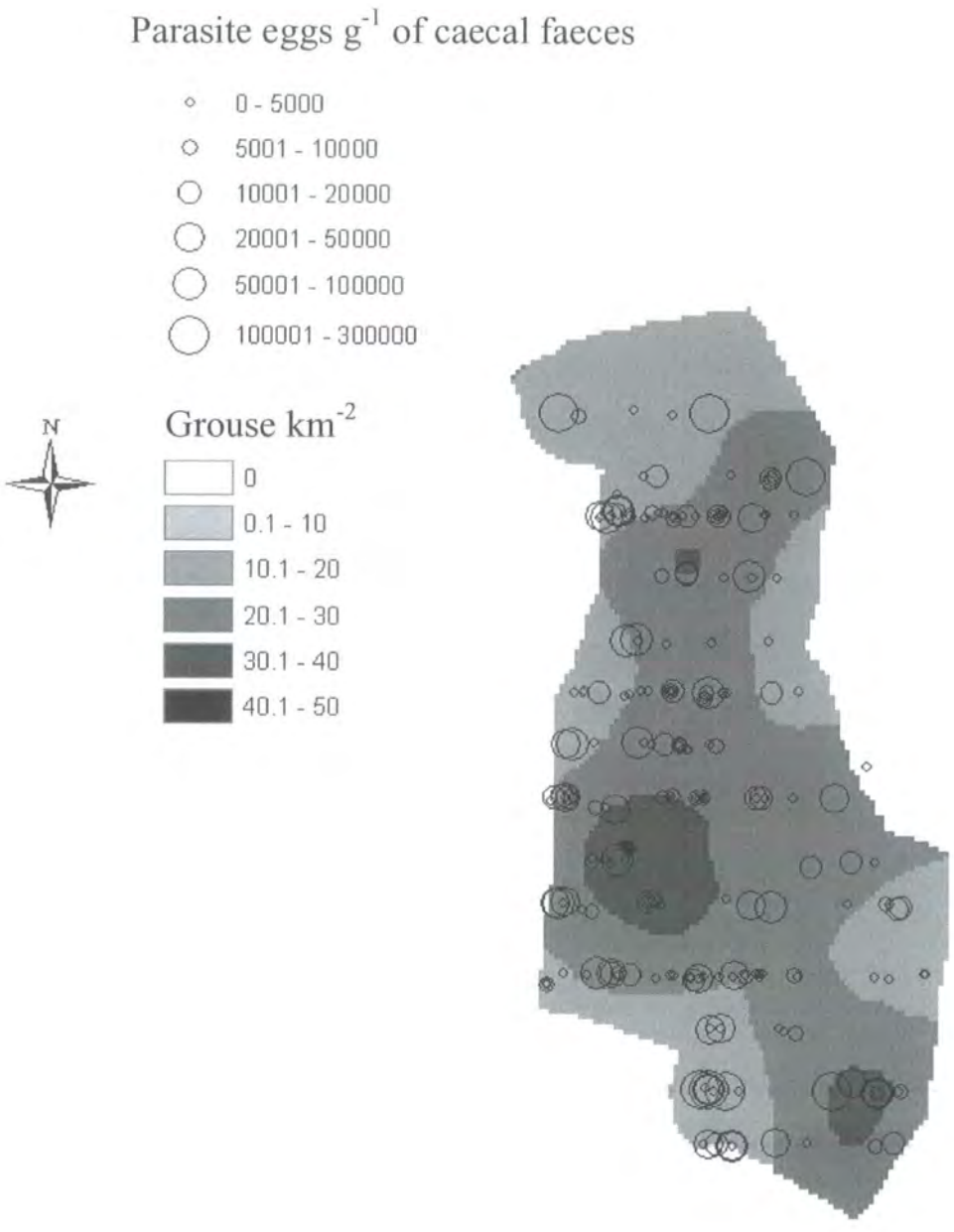
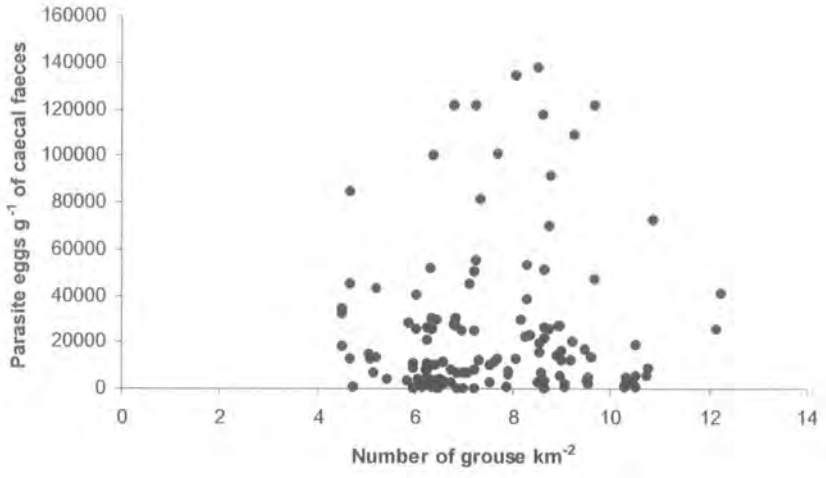


Figure 3.12 Egg concentration in caecal faeces compared to grouse density on Eggleston moor in July. Grouse density map reproduced with permission of P. Warren, Game conservancy Trust.

a



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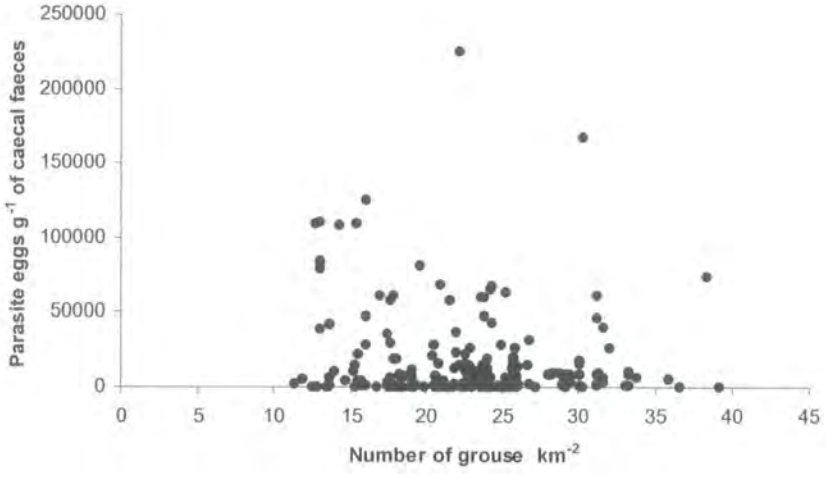

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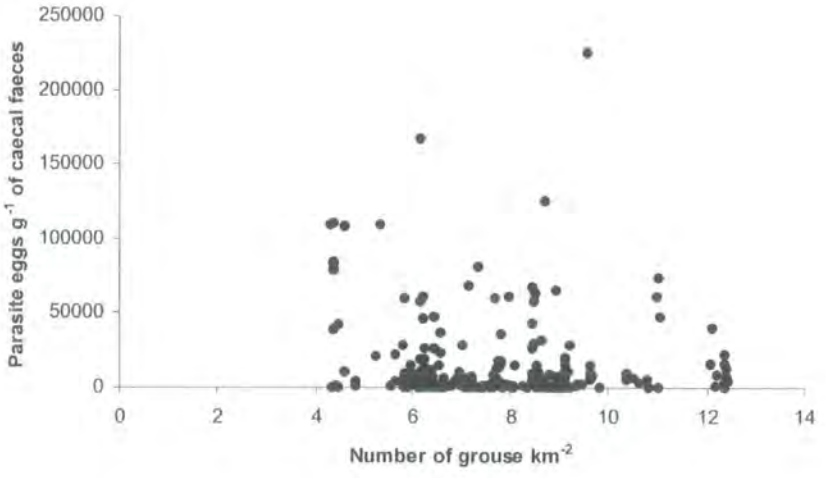


Figure 3.13 Egg concentration in caecal faeces compared to grouse density. a:eggs and grouse in April; b: eggs and grouse in July; c: eggs in July and grouse in April.

3.4 DISCUSSION

3.4.1 Distribution of parasites in the sample of birds

I found *T. tenuis* infection to be prevalent on Eggleston moor: all birds sampled (n=55) had parasites and 93.3% (361/387) of the caecal samples collected over the course of the study contained parasite eggs. This extraordinary rate of infection has been supported by other studies on this species (Hudson 1986a; Wilson 1983). However despite the virtually ubiquitous infection rate in this grouse population, the distribution of the parasite burden among individuals was not uniform. Like many parasites (for review see Shaw & Dobson 1995) *T. tenuis* were aggregated in the red grouse population. The degree of aggregation was similar to that recorded in other grouse populations where the mean value of the negative binomial k=2.85 (Hudson *et al.* 1992b). Aggregation in *T. tenuis* populations is actually comparatively low in relation to many parasites (where k of the negative binomial is often less than 1) (e.g. Anderson & May 1978; Shaw & Dobson 1995). This may partly be accounted for by the high prevalence and intensity of infection (Anderson 1978). The degree of aggregation has important influences on the host parasite population dynamics, with highly aggregated parasites ($k<1$) tending to stabilise host parasite interactions. This occurs because parasite-induced losses from the host population are small, since only a few hosts are heavily infected (Anderson & May 1978). However, relatively low levels of parasite aggregation are likely to destabilise the host population. The aggregation of *T. tenuis* in red grouse observed here fits the latter pattern, supporting the hypothesis that this parasite can destabilise red grouse numbers and generate cycles in red grouse populations (Dobson & Hudson 1992).

The aggregation of parasites among the host population is determined by a variety of factors, which include heterogeneities in host populations or in infection pressure derived from differences in host behaviour or physiology. Spatial variability in exposure to infection can also crucially influence the degree of aggregation (Anderson & Gordon 1982; Grenfell *et al.* 1995). I found that two variables explained much of the variation in the parasite burden in the sample of dead grouse. Firstly, adults had more parasites than young birds, an observation that has been recorded in other red grouse populations (e.g. Wilson 1983; Shaw & Moss 1989a). Quantification of parasite burdens on other moors has shown that the few uninfected birds were all less than 2 months old (Hudson

1986a). This implies that young have not yet ingested infective larvae: chicks must encounter and consume a piece of heather bearing infective larvae before becoming infected themselves. The low levels of infection among young birds is likely to explain the small number (6.7%) of uninfected caecal on the moor; indeed, 25 of the 26 caecal samples were collected in July, shortly after chick hatching. Parasite burdens increase as birds' age because red grouse have little or no immunity against the parasite (Hudson 1992, Shaw and Moss 1989b; Hudson & Dobson 1997), and because *T. tenuis* are long lived (Shaw & Moss 1989a). A limitation of the present study is that I was unable to measure the age of birds – obviously it is impossible to determine the age of the bird that produced a particular caecal faeces. However, it should be recognized that the range of parasite infection levels recorded in caecal faeces on the moor could be partly influenced by the age of the host. Secondly, the position on the moor interacting with bird age did influence parasite burden in the sample of dead birds. This may result from a difference in the availability of larvae in previous years, which would be most likely to result from either environmental conditions or grouse density (see below). Certainly environmental conditions appear to differ broadly from north to south – the north of the moor is mostly blanket bog, while the south is much dryer (P. Warren pers comm.). Free-living parasites are more likely to survive in humid areas (Watson 1988; Shaw *et al.* 1989b) and they also require moisture for migration (McGladdery 1984). Environmental conditions on blanket bog are therefore more likely to be favourable for free-living parasite survival and transmission.

3.4.2 Parasite eggs in caecal faeces

I demonstrated that adult parasite burden could be predicted from caecal egg concentration for burdens up to 6020 (other authors have demonstrated a relationship up to 8000 parasites; Seivwright *et al.* 2004). The residual variation in egg production may partly be due to the range of ages of the worm population in the host, since egg production decreases as parasites age (Shaw & Moss 1989a). Parasite egg production was not affected by intensity of parasite infection in the host, in agreement with previous studies, which have rejected the hypothesis that egg production is density-dependent (Shaw & Moss 1989a; Moss *et al.* 1993b; Hudson & Dobson 1997). The mean egg production I recorded (61.46 eggs parasite^{-1} g^{-1} of caecal faeces) was considerably greater than previous estimates of around 10 eggs worm^{-1} g^{-1} (Hudson *et al.* 1992b), although this is likely to be due to the small sample size in this study. In

addition it may be the result of a seasonal decline in egg production in winter (Shaw & Moss 1989a) as well as with ageing of the parasite population as described above.

Like the adult parasite population, egg concentration was aggregated among caecal faeces collected from the moor (this was also supported in the caecal sampled directly from dead birds). The concentration of eggs in caecal faeces provides an indication of the abundance of infective larvae available to infect future generations of grouse. As discussed earlier, exposure to infective stages is likely to influence the aggregation of adult parasites in birds, and the aggregation of parasite eggs in faeces supports the assumption that encounter rates with infected heather varies spatially. However despite this significant aggregation of parasite eggs, there was only a weak spatial component to the parasite concentration of individual caecal faeces. Thus the parasite burden in one sample was largely independent of the parasite burden in those caecal faeces located nearby. On the basis of my data it seems that there is no spatial clumping of parasite infection at the resolution used here. More intensive sampling would reveal more about the spatial distribution of parasite eggs, and it may be that clumping occurs at a much finer scale (the transects I used were located 500m apart, whereas the home range size of an individual grouse can be as small 10000m^2). Alternatively, intrinsic factors may influence local infection rate – these are further discussed below.

One factor which might influence the local abundance of parasite eggs is the spatial relationship with host density (Anderson 1982). Other studies, using a non-spatial approach, have found that prevalence of *T. tenuis* is consistently extremely high irrespective of host density (Shaw & Moss 1989a; Moss *et al.* 1993b). Given this consistently high prevalence of infection, there is only limited scope for a spatial correlation between host density and degree of infection. I nevertheless found that egg concentration was weakly related to local grouse density in April, although no such relationship existed in July. The presence of caecal faeces from young birds in July with few parasites may have confounded the results for this month, but for the April sample it seems that the expected increase in egg production with increased host density holds, if only weakly.

The relationship between host and free-living parasite density contributes to understanding host parasite population dynamics. Parasites can theoretically regulate

host population numbers when transmission is density-dependent. If there is a time lag between host density and the detrimental effects of the parasite on the hosts, then host and parasite numbers may cycle (Anderson & May 1978). It was not a primary aim of this study to test for a time lag in the relationship between host density and parasite burden, and I was unable to find any correlation between caecal egg concentration in July and host density in the previous April. However a relationship may exist at longer time intervals, and other studies have supported this. For example, long-term studies in England have found that parasite burden was correlated with density of grouse in the July of the previous year (Hudson *et al.* 1992b). In comparison, studies in Scotland showed that grouse density was only weakly related to caecal egg concentration, and that rainfall in the previous summer explained the majority of variation in egg counts between years, probably because parasite recruitment was greatest during wet summers (Moss *et al.* 1993b). The studies therefore drew different conclusions: the parasite could play a role in population cycles on the moor in northern England, yet was unlikely to cause cycles on the study site in Scotland. Differences between the studies may at least in part be explained by differences in regions, since the site in northern England was wetter and grouse densities and worm burdens were higher than the study site in Scotland.

Spatial location on the moor and host density did not explain much of the contemporary variance in egg concentration in caecal faeces, but there are many other unmeasured factors which could influence infection. For instance, the type of vegetation could be considered one important influence on transmission. Indeed migration of larvae may be influenced by chemical cues from heather plants or vertical structures for larvae to ascend (Saunders 1999). In addition, influence of microclimate on free-living parasites is important to their development and survival. A study on a Scottish grouse moor showed that egg counts varied between localities: this study eliminated ground moisture content and proportion of heather to grass as explanatory variables (Moss *et al.* 1993b). Alternatively there may be individual differences between birds in their response to infection. In support of this is the observation that individual differences in egg production persist across years, so that relatively high or low egg counts are to some extent characteristic of individual birds. The reasons for this are unclear; suggestions include differences in interannual worm survival, differences in susceptibility of

individual birds to infection (Wilson 1983), to consistent differences in exposure to infective larvae, or to other environmental differences (Moss *et al.* 1993b).

3.4.3 Conclusion

I found that the parasite *T. tenuis* is extremely prevalent on Eggleston moor, both among dead birds and in caecal faeces collected directly from the moor. In common with many parasites, and with other populations of this parasite, both adult parasites and eggs were aggregated, so that a small number of animals (and faeces) contained the largest parasite burdens. However this degree of aggregation is relatively low compared to other parasite species, and this may be an important factor in the cycling of red grouse populations. Other factors may also influence the host-parasite relationship, and in particular I examined the effect of spatial distribution. There was a north-south difference in the level of infection among adult birds, but only limited spatial autocorrelation in the egg concentration among individual faeces. Similarly the effect of grouse density had only a limited effect on egg production. This emphasises a likely role for intrinsic factors (e.g. birds testosterone level or resistance to parasitism) in determining the individual infection rate of grouse, although detailed sampling at finer spatial scales could reveal more about parasite egg distribution. These data are useful in understanding the nature of the relationship between grouse and the associated parasite population. For example, the aggregated distribution of parasites among hosts and among caecal faeces indicates that models based on spatially uniform distribution of infective parasites could be unrealistic, and emphasises the need for spatial data in interpreting the host-parasite relationship.

CHAPTER 4

Modelling the interaction of red grouse and the parasite *Trichostrongylus tenuis.*

ABSTRACT

Mathematical modelling has shown that parasites are capable of causing host population cycles (Anderson & May 1978; May & Anderson 1978). The parasite *T. tenuis* may be responsible for population cycles in many red grouse populations. I adapted Anderson and May's models to examine the effects of certain parasite-related parameters on the host population. Following this I developed an individual-based stochastic model, which specifically modelled the red grouse-*T. tenuis* interaction. I used this to demonstrate that *T. tenuis* can regulate red grouse populations although I was unable to find any parameter combinations that led to host population cycles.

4.1 INTRODUCTION

4.1.1 Red grouse population cycles

Early descriptions of red grouse shooting records ('bag records') from the British Isles (Middleton 1934; Mackenzie 1952) showed that many red grouse populations tend to fluctuate in numbers over time (Moran 1952; Williams 1985). Later analysis demonstrated population cycles in 58% of English records and 77% of Scottish records (173 time series analysed; Hudson 1992). A more recent paper suggested that 63.3% of populations (of a total of 289) showed population cycles (Haydon *et al.* 2002). The majority of cycles are 'phase-forgetting' cycles (Nisbet & Gurney 1982), which tend to drift out of phase (Potts *et al.* 1984; Hudson 1992; Haydon *et al.* 2002). The pattern of red grouse cycles varies between populations. Cycles with periods of around 3 to 5 years (Williams 1985; Potts *et al.* 1984; Hudson 1992; Hudson *et al.* 2002) and up to 15 years have been recorded (Hudson 1992; Haydon *et al.* 2002). In general, populations in northern Scotland have significantly longer periods than those in northern England (Hudson 1992; Hudson *et al.* 2002) although only a small amount of variation (5.3%) has been attributed to latitude, with most being due to complex regional effects (Haydon *et al.* 2002). Amplitude of cycles varies greatly between grouse populations (Lambin *et al.* 1999) and can vary from 3 to 10 fold (Watson *et al.* 1984; Moss & Watson 2001). Cycles in northeast Scotland are typically symmetrical, with increase and decline phases approximately equal in length (Moss *et al.* 1996) compared to fluctuations in populations in northern England which are characteristically asymmetrical, with long periods of increase followed by sudden crashes (Hudson *et al.* 1992a; Hudson *et al.* 1998).

The cause of red grouse cycles is of interest both theoretically and because severe decreases in grouse numbers result in loss of revenue for estates (Hudson 1992). Cycles in animal populations are thought to be primarily caused by density-dependent regulation of the population acting with a time delay (May 1981). Growth of a population is limited for example by resources and levels off at the carrying capacity. Numbers will be stable when the proportional loss from the population increases with density. However numbers can become unstable and fluctuate if there is a time delay in

the response of the population to a certain factor such as environmental conditions or a change in food availability. In certain conditions, this can result in population cycles.

There are two main hypotheses to explain population cycles in red grouse. Firstly the kin facilitation hypothesis proposes that delayed density-dependent changes in aggression influence spacing behaviour and the rate that males are recruited into the breeding population, therefore leading to cycles in numbers (Mountford *et al.* 1990; Watson *et al.* 1994; Moss *et al.* 1996; Matthiopoulos *et al.* 1998, 2000; MacColl *et al.* 2000; Moss & Watson 2001). Secondly, cycles might be generated by parasite-induced reductions in host breeding and survival (Hudson *et al.* 1985, 1992a, 1998; Hudson 1986b, 1992; Dobson & Hudson 1992). This chapter will focus on the hypothesis that *T. tenuis* can generate population cycles in red grouse.

4.1.2 Parasite-induced population cycles

Parasites can have a detrimental effect on the fecundity and survival of their hosts in wild populations (for review see Tompkins & Begon 1999) and have been implicated in natural population cycles (e.g. in red fox: Anderson *et al.* 1981; Soay sheep: Gulland 1992; snowshoe hares: Ives & Murray 1997 and insects: Reeve *et al.* 1994). In red grouse populations, *T. tenuis* has a detrimental effect on red grouse survival and breeding (Potts *et al.* 1984, Hudson 1986b, Shaw *et al.* 1990, Hudson 1992, Hudson *et al.* 1992b; Newborn & Foster 2002). The importance of *T. tenuis* in regulating grouse populations has been demonstrated by long term experimental treatment of grouse (to reduce parasite infections), which reduced the magnitude of cyclic population declines (Hudson *et al.* 1998). Although this demonstrated the importance of *T. tenuis* in red grouse regulation, the populations continued to show cyclic fluctuations (see Lambin *et al.* 1999).

Manipulative experiments on wild animal populations are an important method of exploring the role of parasites in population regulation; a complimentary method to gain insight into their population dynamics is through modelling. Theoretical mathematical modelling by Anderson and May (1978) and May and Anderson (1978) has shown that parasites can cause population cycles in their hosts if they affect survival or reproduction in a density-dependent manner. Their models were subsequently used to

study red grouse – *T. tenuis* population dynamics (Dobson & Hudson 1992). However several aspects of the red grouse *T. tenuis* system remain to be modelled explicitly using this method.

A further method of modelling the host-parasite relationship is through individual-based models. These represent interactions between the host and parasite at the level of the individual, and any population-wide effects that result are emergent properties of these individual interactions. Simulation models based on discrete individuals have the advantages of clarity and realism (Mollison & Levin 1995) and can be more appropriate than deterministic models that treat the population changes as a continuous process (McCallum & Scott 1994; Rushton *et al.* 2000). This type of model has not previously been used to study the relationship between red grouse and *T. tenuis*. Design and parameterization of such a model is likely to be relatively accurate for the red grouse-*T. tenuis* system, as both the host and parasite have been the subject of intensive study resulting in a large body of detailed literature.

4.1.3 Aims

This chapter is divided into two sections: Section one describes basic host parasite dynamics using mathematical models designed by Anderson & May (1978) and May & Anderson (1978). I use these models to examine the effects of parameters that influence changes in the parasite population, on the host population. The aim of section 2 is to design and analyse a stochastic individual-based model, specifically describing the red grouse - *T. tenuis* interaction, to assess whether *T. tenuis* are capable of generating cycles in the size of the host population.

4.2 SECTION 1 – DETERMINISTIC MODELS

The host-parasite population models of Anderson and May (1978) and May and Anderson (1978)

4.2.1 INTRODUCTION

Anderson and May (1978) and May and Anderson (1978) described a series of mathematical models based on biological features of host-parasite associations. These demonstrated mathematically for the first time that parasites could cause host population cycles. The models have subsequently been used widely in host-parasite research (the two papers were cited more than 600 times between 1980 and 2004). Their first model (the 'basic' model) considers parasite-induced mortality on a host population. Various modifications of this model showed that certain biological processes (e.g. over-dispersion of parasites among hosts, time lags in parasite development) can have either a stabilising or a destabilising effect on the host population. By modifying the parameters in these models on the basis of real parasite and host populations, they can be used to understand the dynamics of specific interactions. Here I describe two of the model variants described by Anderson and May, before developing them as specific models of the red grouse-*T. tenuis* system. The first of these, the basic model, represents the simplest host-parasite interactions and is useful for outlining the fundamentals of the system. A more sophisticated model, model F, includes a time delay in parasite development and is likely to most accurately represent the red grouse – *T. tenuis* system.

The basic model (Anderson & May 1978)

The basic model represents the interaction of a host population and a direct life cycle parasite that produces transmission stages that develop outside of the host (such as eggs). Parasite-induced mortality is linearly proportional to the number of parasites in the host. Two differential equations represent the change in the host (H) (Equation 1) and parasite (P) (Equation 2) populations. Table 4.1 describes the model parameters.

$dH/dt = (a-b)H-\alpha P$ Equation 1

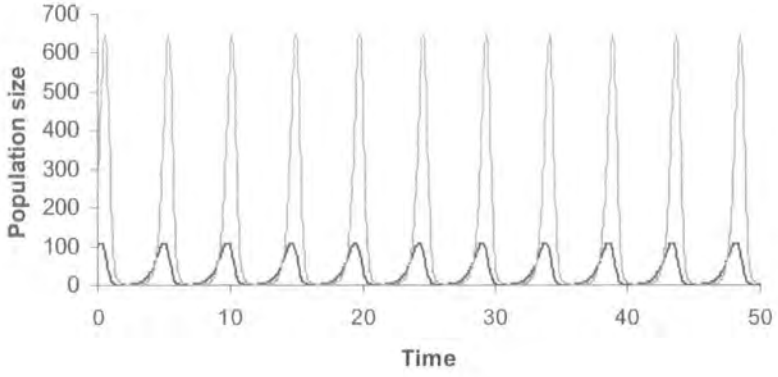
$dP/dt = P(\lambda*H/(Ho + H) - (b +\mu +\alpha)-\alpha*P/H)$. Equation 2

Table 4.1 Description of population parameters used in the basic model (from Anderson & May 1978).

Parameter	Description
a	Instantaneous host birth rate (host^{-1} unit time^{-1})
b	Instantaneous host death rate, mortalities due to natural causes (host^{-1} unit time^{-1})
α	Instantaneous host death rate, mortalities due to the influence of the parasite (host^{-1} unit time^{-1})
λ	Instantaneous birth rate of parasite transmission stages where birth results in the production of stages which pass out of the host and are responsible for transmission of the parasite within the host population (parasite unit time^{-1})
μ	Instantaneous death rate of parasites within the host, due to either natural or host induced (immunological) causes (parasite^{-1} unit time^{-1})
Ho	Transmission efficiency constant, varying inversely with the proportion of parasite transmission stages which infect members of the host population

Anderson & May (1978) demonstrated that the parasite regulates the host and causes host population cycles under certain conditions. If the growth of the host population is positive ($a-b>0$) then the parasite causes cycles when the birth of the parasite transmission stages is high (specifically $\lambda>\mu+\alpha+a$), (Figure 4.1a). The parasite population peaks after the host population begins to decline. When birth of transmission stages is low (specifically if $\lambda<\mu+\alpha+a$) the parasite cannot establish in the host in great enough numbers to have a regulatory effect and the host population grows exponentially. The parasite population also grows exponentially but at a slower rate than the host population and the mean number of parasites per host tends to zero (Figure 4.1b). When cycles occur, the period depends on the model parameter values and the amplitude depends on the initial conditions of the displacement. The cycles are neutrally stable, meaning they continue indefinitely if undisturbed. A shift in parameter values initiates a different neutrally stable cycle, around the same mean but with different amplitude. In real populations external influences would continually shift the cycles to new values and therefore the authors concluded that the model was unrealistic.

a



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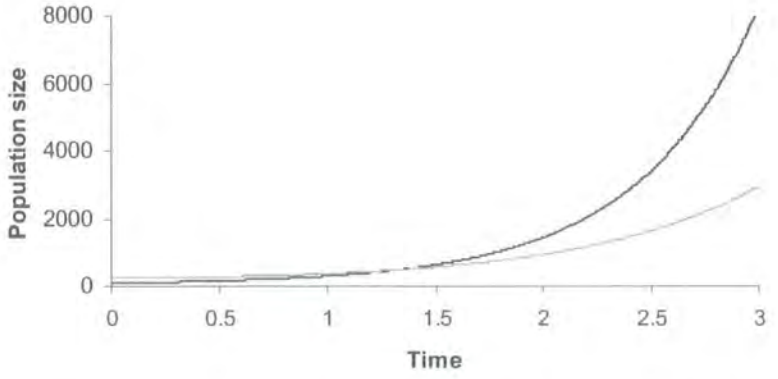


Figure 4.1 Host and parasite population dynamics generated by the basic model by Anderson & May 1978. Host (black) and parasite (grey). a. Populations cycle when parasite birth rate is high. Parameter values: a=3, b=1, α=0.5, μ=0.1, Ho=10, λ=6. b. Exponential growth of host and parasite populations when parasite birth rate is low. Parameter values: as figure a except λ=3

Model F (May & Anderson 1978) - The influence of time delays in parasite transmission

The basic model assumes immediate transmission of the parasite to the host, however in many parasite life cycles there is a time delay in transmission between the birth of the transmission stage and infection of a new host. For example, *T. tenuis* eggs take a minimum of 9 days in optimum conditions to develop to infective larvae (Shaw *et al.* 1989) which once ingested by a host take approximately 12 days to become mature adults (Shaw 1988). May and Anderson (1978) incorporated the influence of time delays in transmission using a third differential equation in the basic model to represent

the population of free-living parasite stages (W) (Equation 3F below) (May & Anderson 1978). All parameters are described in table 4.1 with additional parameters in table 4.2.

$dH/dt = (a-b)H - \alpha P$ Equation 1

$dP/dt = \beta WH-(\mu+\alpha+b)P-\alpha (k+1)P^2/(kH)$ Equation 2F

$dW/dt = \lambda P-\gamma W-\beta WH$ Equation 3F

Change in the host population remains the same as basic model (Equation 1). The parasite population equation (Equation 2) is modified so that the parasites were aggregated within the host population, (Equation 2F). This reflects the distribution of parasites that often occurs in real host populations, where only a few hosts are infected with a high proportion of the parasite population. Change in the free-living parasite population depends on birth, death and transmission to the host (Equation 3F).

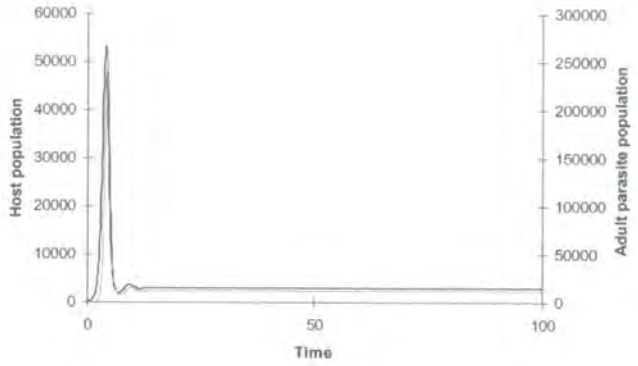
Table 4.2 Description of population parameters used in model F in addition to those described in table 5.1.

Parameter	Description
γ	Instantaneous rate of loss of parasite infective stages (due to death or other processes that prevent them infecting a host) (unit time^{-1})
β	Instantaneous rate of ingestion of parasite infective stages (host^{-1} unit time^{-1})
k	Parameter of the negative binomial distribution which measures inversely the degree of aggregation of parasites within the host population

May & Anderson (1978) showed that time delays in parasite transmission have a destabilizing effect on host dynamics. If the birth of the transmission stages is low ($\lambda<d$, where $d=\mu+\alpha+b+(a-b)(k+1)/k$) then the parasite cannot regulate the host population, which grows exponentially, and host parasite burdens decrease to low values. The parasite can establish in the host population when the parasite has a very high reproductive rate (specifically $\lambda>d$). The stability of the host-parasite interaction depends on the life expectancy of the free-living parasite (γ) and on the aggregation of

the parasite within the host (k). A further equation describes the conditions for stability of the equilibrium: $d/\gamma <= 1/k$. When the condition is satisfied the host and parasite populations are stable (Figure 4.2a). Host and parasite population cycles occur if the condition is not satisfied. The tendency for the system to cycle increases as free-living parasite mortality decreases. When larval mortality is high the population initially fluctuates before reaching stability (Figure 4.2b $\gamma=0.1$); in comparison with lower larval mortality where the populations cycle (Figure 4.2c $\gamma=0.01$). The host and parasite interaction becomes more stable if parasite aggregation increases (k decreases). When parasites are highly aggregated host populations are unlikely to cycle because parasite-induced mortality only occurs to those hosts with high parasite burdens.

a



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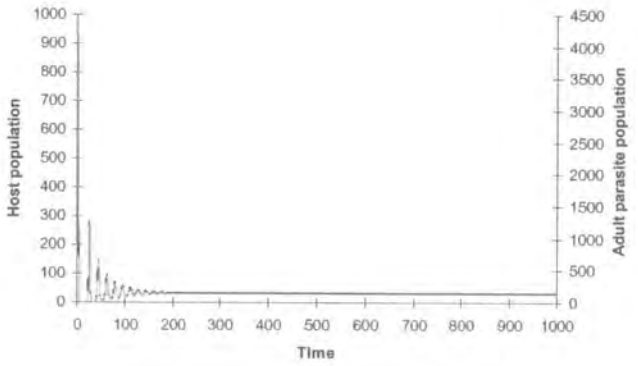

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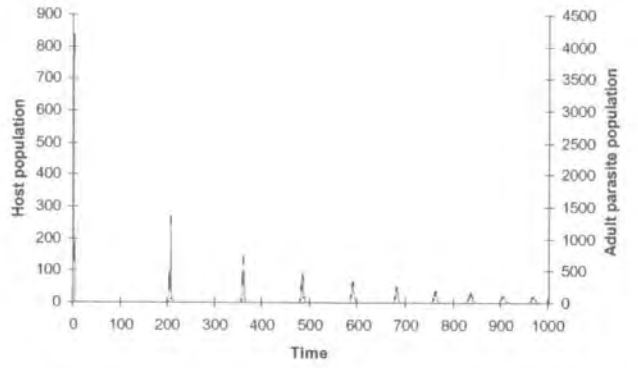


Figure 4.2 Host and parasite population dynamics generated by model F by May & Anderson 1978. Host (black) parasite (grey). a. Host and parasite reach stable values when the mortality of parasite infective stage is high (γ=9.2). Parameter values: a=3, b=1, α=0.5, μ=0.1, λ=6, k=2, β=0.01, γ=9.2. b. Host and parasite populations cycle and eventually reach equilibrium when the mortality of parasite infective stages is low (γ=0.1). Parameter values as figure a except γ=0.1. c. Host and parasite populations cycle when the mortality of parasite infective stages is very low. Parameter values as figure a except γ=0.01. Host and parasite decrease to very low values (approximately $1x10^{-33}$).

Application to red grouse and T. tenuis.

Dobson & Hudson (1992) used a modified version of May and Anderson's model F to demonstrate that *T. tenuis* could theoretically cause cycles in grouse populations through its detrimental effect on host survival and reproduction. The model consists of three differential equations (Equations 1D&H, 2F and 3F). The original equation describing change in the host population (Equation 1F) was modified to include a parameter (δ) representing the instantaneous reduction in grouse fecundity due to the parasite (Equation 1D&H). Equations describing the change in adult parasite (Equation 2F) and free-living parasite (Equation 3F) remain the same as model F.

$$dH/dt = (a-b)H - (\alpha+\delta)P \qquad \text{Equation 1D\&H}$$

$$dP/dt = \beta WH-(\mu+\alpha+b)P-\alpha\,(k+1)P^2/(kH) \qquad \text{Equation 2F}$$

$$dW/dt = \lambda P-\gamma W-\beta WH \qquad \text{Equation 3F}$$

Sustained cycles in host abundance do not occur when parasite induced reduction in grouse breeding is absent, because infected hosts die quickly (in contrast to the Anderson & May model, which did cause host cycles without parasite-induced reduction in host breeding). The parameters used in the grouse model did not meet the requirements for cyclic dynamics (section 4.2.1). When parasite effects on breeding were included (and were greater than the effect on grouse mortality) the host numbers cycled, although these cycles were unstable and led to extinction of the host and parasite population. In reality, oscillations would be limited at high density by other mechanisms and therefore the authors included a density-dependent factor to reflect territorial defence (grouse fecundity decreased with increasing density). Without parasite induced affects on grouse breeding host and parasite showed damped cycles, which reached stable values. Inclusion of parasite affects on breeding caused weakly damped or diverging cycles of host and parasite abundance.

The period of the cycles was determined by the life expectancy of the free-living larvae (in agreement with May & Anderson (1978)). The parasite established in the host population when larval life expectancy was 2-4 weeks. As larval life expectancy increased, the period of host and parasite population cycles increased. In addition as the natural growth rate of the host population increased the period of the cycles decreased. Further modifications to the model showed that short periods of larval arrestment (2-3 months) increased the tendency for the populations to cycle and also increased the period of the cycles compared to the first model. Longer periods of arrestment (more than 6 months) resulted in damped cycles and eventually to stable host and parasite populations (no oscillations).

Host population cycles produced by the first model were characteristic of grouse populations in northern England (Potts *et al.* 1984; Hudson *et al.* 1992b), with an asymmetrical shape (slow host population growth followed by a rapid decline) and a period of 4 to 5 years. Parasite burdens only peaked after the host population had started to decline, an effect has been seen in real populations in Gunnerside, north Yorkshire (Hudson *et al.* 1992b). The model including arrested larval development produced cycles with a period of 7-10 years which were similar to cycles that have been recorded in Scotland (Mackenzie 1952; Williams 1985).

4.2.1.1 Aims

Although the models of Anderson and May have previously been applied to the red grouse *T. tenuis* system (Dobson & Hudson 1992), several parameters of specific relevance to this interaction have not yet been fully explored. Rather than expanding directly on Dobson and Hudson's work I returned to the original models by Anderson and May to examine some of these parameters. A specific limitation of the original models is that they are described by differential equations, and hence operate in continuous time. In comparison, in some populations, growth takes place at discrete time intervals. For example reproduction in red grouse takes place over a discrete time interval once per year. In this section I modify the original models to investigate the effects of parameters likely to be of importance in this system, before recasting the models in discrete time.

4.2.2 METHODS

Models in continuous time

I began by studying the sensitivity of the original and most simple model of hosts and parasites i.e. the 'basic' model (Anderson & May 1978). I then continued by assessing the sensitivity of certain parameters in model F (May & Anderson 1978). The parameters that were manipulated were not specifically studied in as much detail by the original authors, and have been chosen here because they influence the rate of change of the parasite population. The basic model and model F were run using Modelmaker (version 3, Cherwell Scientific Publishing Limited, Oxford). Initial conditions were set following the original publications, with 100 hosts, 100 free-living parasites and 250 adult parasites. Models were run from time=0 to 1000 with the host and population size reported each 0.1 of a time unit.

Basic model

The influence of two parameters: the birth of parasite transmission stages (λ) and the transmission efficiency constant (Ho) were varied in two simulations. Both parameters influence the rate of change of the parasite population in Equation 2. Values of Ho were varied from 6 to 12 and values of λ from 4 to 15. These values were chosen because they result in cyclic dynamics of the host and parasite population (according to the equation: $\lambda<\mu+\alpha+a$ (section 4.2.1)). Other parameter values were constant within the range specified by Anderson & May (1978) and were altered between simulations 1 and 2; (Simulation 1: a=3, b=1, α=0.5, μ=0.1, λ=4-15, Ho=6-12; simulation 2: a=5, b=2, α=0.8, μ=0.2, λ=7-15, Ho=6-12). The period of cycles in time series produced from each simulation was measured and the effect of Ho and λ determined.

Model F

I examined the influence of free-living parasite mortality (γ) on the host and parasite population equilibrium and on the occurrence and period of cycles. Seventeen values of free living mortality (γ) were tested; (0.005, 0.008, 0.01, 0.02, 0.025, 0.03, 0.04, 0.05, 0.1, 0.5, 1, 3, 5, 7, 9.2, 11, 15). This range included values that would and would not cause host and parasite stability (where $d/\gamma<=1/k$ (section 4.2.1)). Other parameter values were constant within the range specified by the authors (a=3, b=1, α=0.5, μ=0.1,

λ=6, k=2, β=0.01). Using these parameter values the equilibrium will be met when free living parasite mortality (γ) > 9.2.

For each parameter combination, the mean period of the cycle was calculated from the first three peaks in the time series. The mean host and parasite population was calculated between time=500 to 1000. To determine if the population reached equilibrium the mean, minimum and maximum host population size from time 900 to 1000 were calculated. If the minimum or maximum value was less than 1% of the mean host population then the population was classed as reaching equilibrium.

Model F in discrete time

The differential equations describing model F were modified to difference equations (Equations 3, 4 and 5; subscript t indicates a discrete time point). These describe population change from one time step to the next.

$$H_{t+1} = H_t + (a - b)H_t - \alpha P_t \qquad \text{Equation 3}$$

$$P_{t+1} = P_t + (\beta W_t H_t) - (\mu + \alpha + b)P_t - (\alpha (k + 1)P_t^2) / (kH_t) \qquad \text{Equation 4}$$

$$W_{t+1} = W_t + (\lambda P_t) - (\gamma W_t) - (\beta W_t H_t) \qquad \text{Equation 5}$$

A program to run the discrete time model was written in Visual Basic (version 6, Microsoft Corporation, Seattle, Washington, USA). Initial conditions were set the same as May & Anderson (1978), with 100 hosts, 100 free-living parasites and 250 adult parasites. The size of the host, parasite and free-living parasite population was calculated from time t = 0 to 300 with a time step of 1. The program was limited by the size of the host and parasite population and ended if the host reached 0 or 50000 individuals, or the parasites reached 0 or 1000000 individuals.

Parameter values in this model were based on empirical evidence about the red grouse-*T. tenuis* system. Two tests were used to select appropriate parameter values. In test 1 minimum and maximum values were specified for all parameters and the program selected random values within this range at the start of each simulation. A total of

2,000,000 random parameter combinations were tested using this method. In test 2 values for each parameter were specified and every possible combination modelled (a total of 90,000 parameter combinations). Data were only output if the host population persisted to time step 300. In test 2 the day that the program finished was also output.

Parameter values (maximum and minimum for test 1; exact values for test 2) are shown in table 4.3. Parameters representing mortality rates of host and parasite stages (b, α, μ, γ) were selected between 0 and 1 because discrete death rates cannot exceed 1. Red grouse have an average of 8 chicks per year (Jenkins *et al.* 1963; Hudson 1986a; Hudson 1992; Moss *et al.* 1993b) and so the parameter for host birth (a) was set between 0 and 10. Parasite fecundity (λ) was based on an estimate that the number of eggs produced per adult *T. tenuis* per year is 40000 (Hudson 1992). A mean of 30% of eggs develop to infective larvae (Connan & Wise 1994) and the estimated proportion of larvae available for ingestion by a host was 10% in optimum conditions (Saunders *et al.* 1999). Values for parasite fecundity were therefore selected between 0 and 2000 transmission stages per parasite (which are immediately available for ingestion by a host). Values of k were chosen based on the knowledge that the degree of *T. tenuis* aggregation in red grouse is low compared with other parasite species (estimated k=1.2 to 5.8, mean 2.85), (Hudson *et al.* 1992b). Finally the rate of ingestion of infective parasites (β) was difficult to estimate and therefore was set to less than one. Very small values were selected in the second parameter testing method because if β is large the host population could potentially ingest more larvae than are actually available.

Table 4.3 Description of population parameters used in the discrete model

Parameter	Parameter value		Description
	Test 1	Test 2	
a	0-10	2,4,6,8,10	Host birth ($year^{-1}$)
b	0-1	0.01, 0.05. 0.1, 0.25, 0.5	Host 'natural' mortality rate ($year^{-1}$)
α	0-1	0.01, 0.05, 0.1, 0.25, 0.5	Parasite induced host mortality ($year^{-1}$)
λ	0-2000	10, 100, 1000	Birth of parasite infective stages ($parasite^{-1}$ $year^{-1}$)
μ	0-1	0.05, 0.1. 0.5, 1	Adult parasite mortality ($year^{-1}$)
β	0-1	0.00001, 0.0001, 0.001, 0.01, 0.1	Ingestion of parasite infective stages ($host^{-1}$ $year^{-1}$)
k	0-20	0.5, 2.5, 5	Inverse measure of the degree of aggregation of the parasite in the host (parameter of the negative binomial)
γ	0-1	0.05, 0.1, 0.5, 1	Infective parasite mortality ($year^{-1}$)

4.2.3 RESULTS

4.2.3.1 Models in continuous time

The basic model

The birth of parasite transmission stages (λ) had a much greater influence on the mean period of the cycles than the transmission efficiency constant (Ho) (Figure 4.3). Changes in the host infection rate (low infection rate, high Ho, to high infection rate, low Ho) had little influence on the mean cycle period of the host population. In comparison the birth of the parasite transmission stages did influence the host population cycle period. Generally host population cycle period increased as birth of parasite transmission stages increased. The effect of the two parameters on host cycles was consistent when all other parameters were altered (simulation 2, figure 4.3)

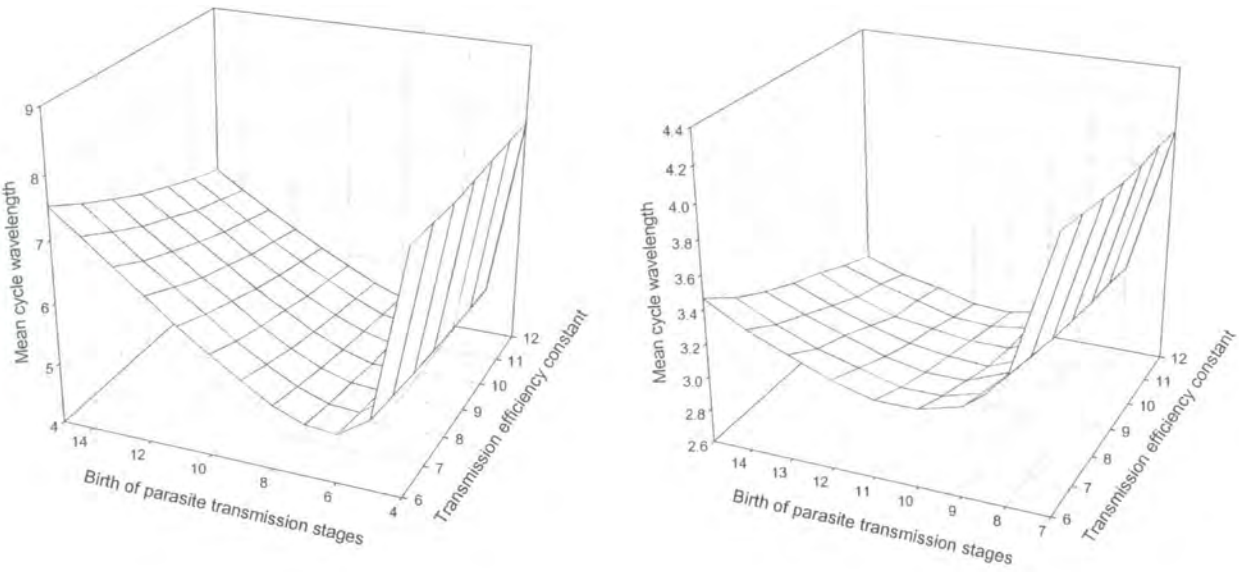
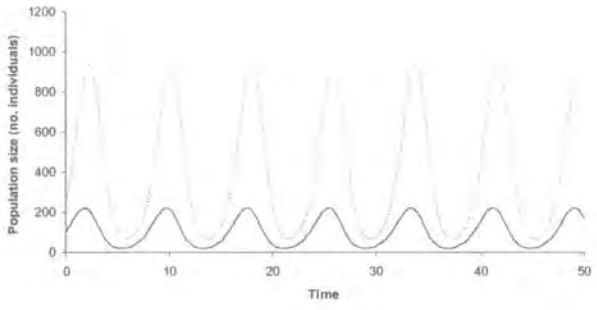
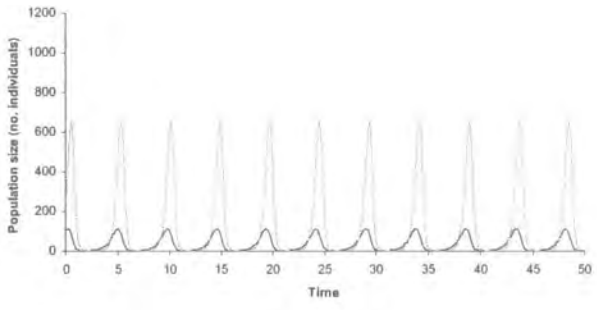


Figure 4.3 The influence of birth of parasite transmission stages (λ) and the transmission efficiency constant (Ho) on the period of host population cycles in the basic model in two simulations
Parameter values: simulation 1 (left) a=3, b=1, α=0.5, μ=0.1, λ=4-15, Ho=6-12; simulation 2 (right) a=5, b=2, α=0.8, μ=0.2, λ=7-15, Ho=6-12.

a



b



c

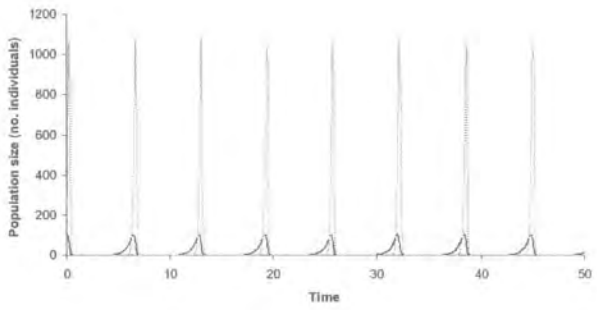


Figure 4.4 Changes in the host and parasite population cycles from the basic model with changes in birth of the parasite tranmission stages. Graphs a, b and c illustrate the host (black) and parasite (grey) population dynamics for increasing rate of birth of parasite transmission stages. Parameter values: a: a=3, b=1, α=0.5, μ=0.1, Ho=6, λ=4; b: as figure a, except λ=6; c: as figure a, except λ=12.

Model F

Host population cycles depended on the mortality of free living parasites (Figure 4.5). Population cycles occurred when infective parasite mortality was low (γ=0.005–0.01) because parasite burdens built up and ultimately caused crashes in the host and consequently the parasite population. Free-living parasites survived long enough to infect individuals when the host population started to recover after a crash. As free-

living mortality increased (γ=0.04–1) the host population cycles dampened to stable values within 1000 time steps. When free-living parasite mortality was high (γ>3) parasite burdens were too low to have a regulatory effect; cycles did not occur and the host population stabilised. The mean host and parasite population size increased as infective parasite mortality increased.

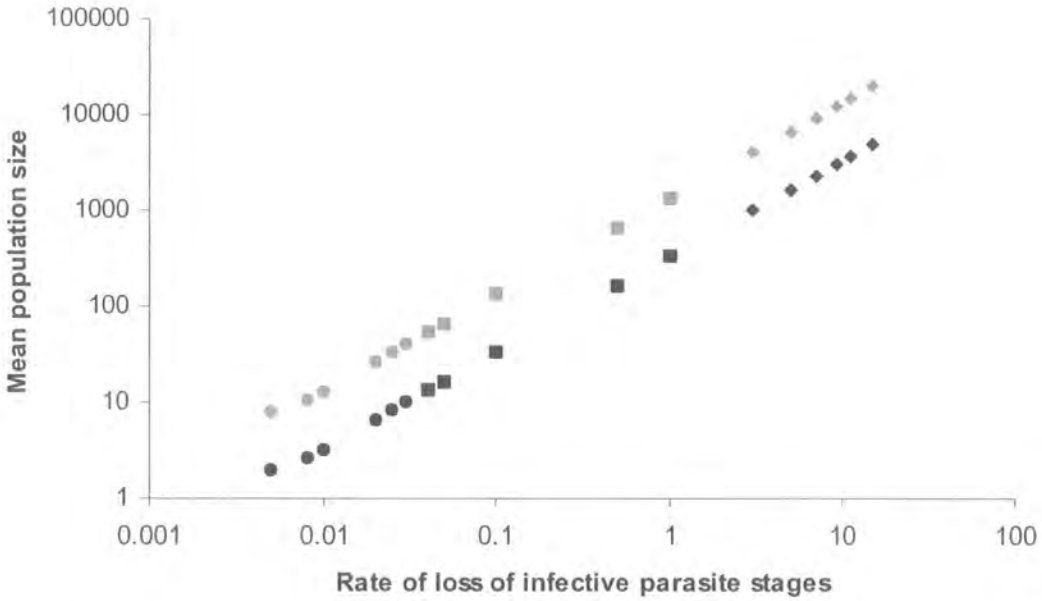


Figure 4.5 The influence of free living parasite mortality on the occurrence of cycles in the host and parasite population in model F. Host (black) and parasite (grey) populations were measured over 300 time units. Circles: population cycles with decreasing period; squares: host and parasite cycles which reached stable values; diamonds: host and parasite did not cycle, values were stable.

In those time series that did cycle, the cycle period decreased as free living parasite mortality increased (Figure 4.6). The period and amplitude of cycles gradually decreased over time, and when parasite mortality was more than 0.04 parasite unit time^{-1} the cycles damped to stable host and parasite values.

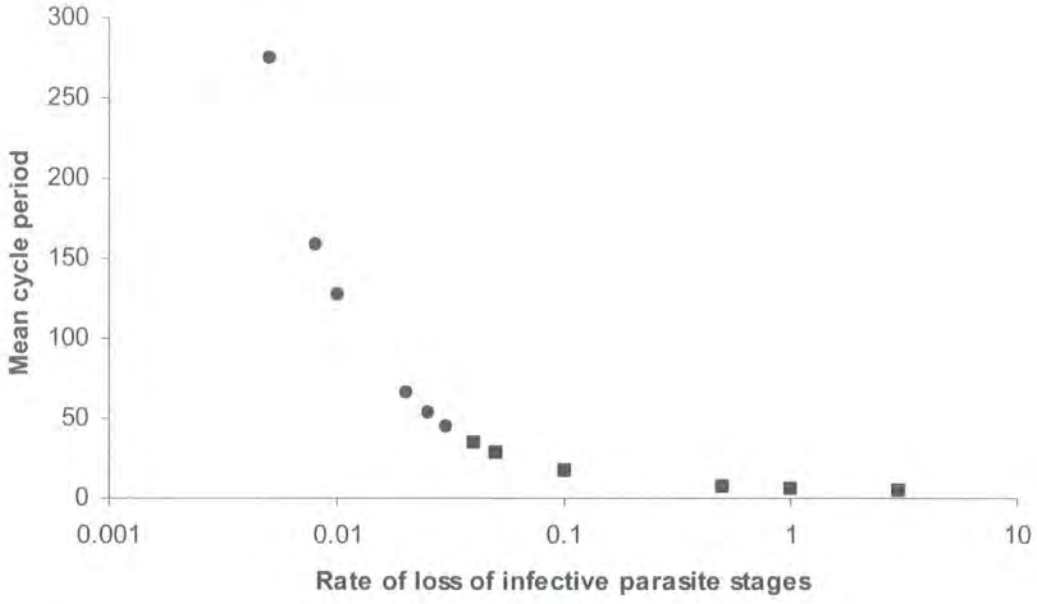


Figure 4.6 The influence of free living parasite survival on the cycle period of host and parasite population according to model F. Host population cycles with decreasing period (circles) or host population cycles that reach stable values (squares) over 300 time units. Mean cycle period was measured from the first three peaks in the time series.

4.2.3.2 Models in discrete time

None of the parameter combinations from either test allowed host population persistence indicating unstable host population dynamics. The maximum time that the host population persisted for in test 2 was 9 time units. These results indicate that stable cycles did not occur. The host population was unstable and all parameter tests finished either because the host population reached the maximum of 50000, or it went extinct. This might suggest mis-assignment of one or more parameters, although the 2000000 randomly chosen parameter combinations represented a comprehensive sampling of the realistic range of values.

4.3 SECTION 2 – INDIVIDUAL BASED MODEL OF RED GROUSE AND *T. TENUIS*

4.3.1 METHODS

4.3.1.1 Model structure

Design and parameterization of the model was based on literature about the red grouse and *T. tenuis*. The model simulates individual red grouse in a population and the associated parasite population. Figure 4.7 illustrates the main events in the host and parasite life cycle that were incorporated in the model. To summarise, adult parasites in the grouse produce eggs that are deposited by the host. Eggs develop to infective larvae which can be ingested by each grouse. Each parasitic stage is subject to daily mortality. Hosts reproduce on one day per year; chicks remain with the parent grouse until they become independent adults. Individual grouse are subject to natural and parasite induced mortality (chicks also die if the parent bird dies). The adult parasite burden within each grouse therefore affects its daily survival. For simplicity immigration and emigration have been excluded.

Host and parasite parameters were chosen based on the available literature (section 4.3.1.3 summarises the parameterization of the model). When a parameter could not be estimated accurately, a range of values was chosen and model simulations run to test all values (section 4.3.2 summarises model sensitivity analysis). This method of sensitivity analysis may be useful for assessing the importance of parameters on the stability and dynamics of the host and parasite populations and may also suggest realistic ranges of parameter values where data were unavailable.

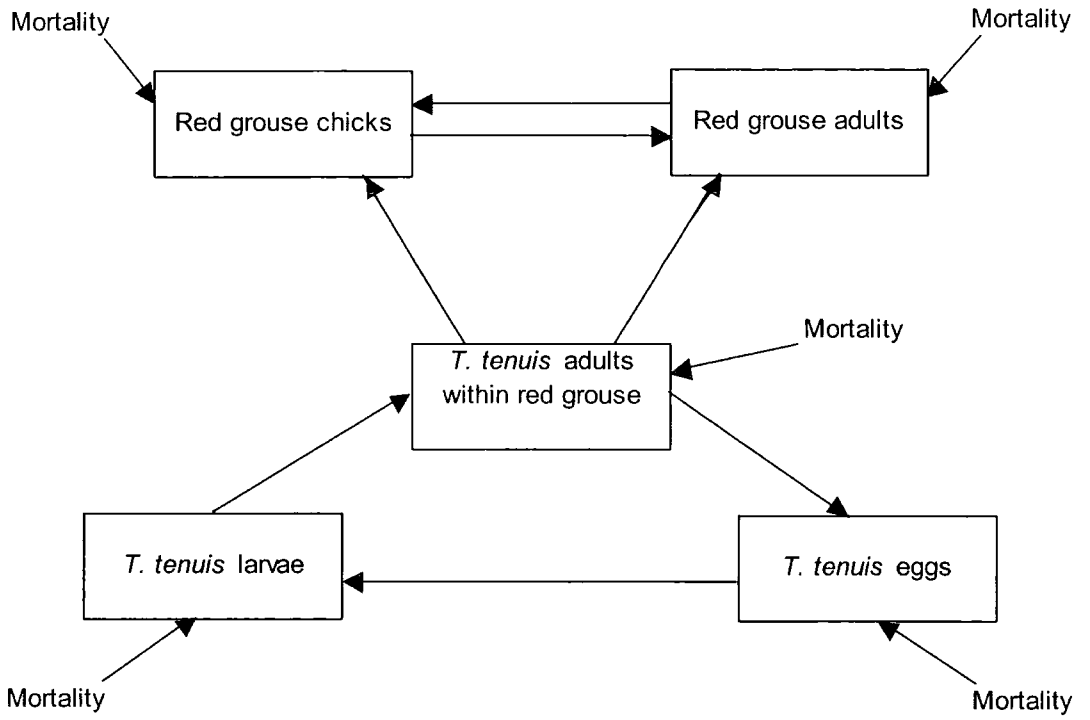


Figure 4.7 The life cycle of the red grouse and the nematode parasite *Trichostrongylus tenuis*

4.3.1.2 Program structure

The program was written in Visual Basic (version 6, Microsoft Corporation, Seattle, Washington, USA). Appendix 1 shows the full program code. Figure 4.8 illustrates the structure of the program. The simulation begins on day one (October 1st) with 100 adult grouse, 100 adult parasites within each grouse, 0 free-living parasite larvae and 0 parasite eggs. The program executes a consecutive set of procedures (Table 4.4) that calculate changes in the host and parasite population each 'model day' using the population size from the previous day. The model was limited by the size of the host population (minimum=0, maximum=30000) and the chick population (maximum=50000). These limits were introduced to prevent the populations reaching unmanageably large levels. The limits were checked at the end of each model day and as long as the requirements were met the program looped to the next day until completing the specified number of years.

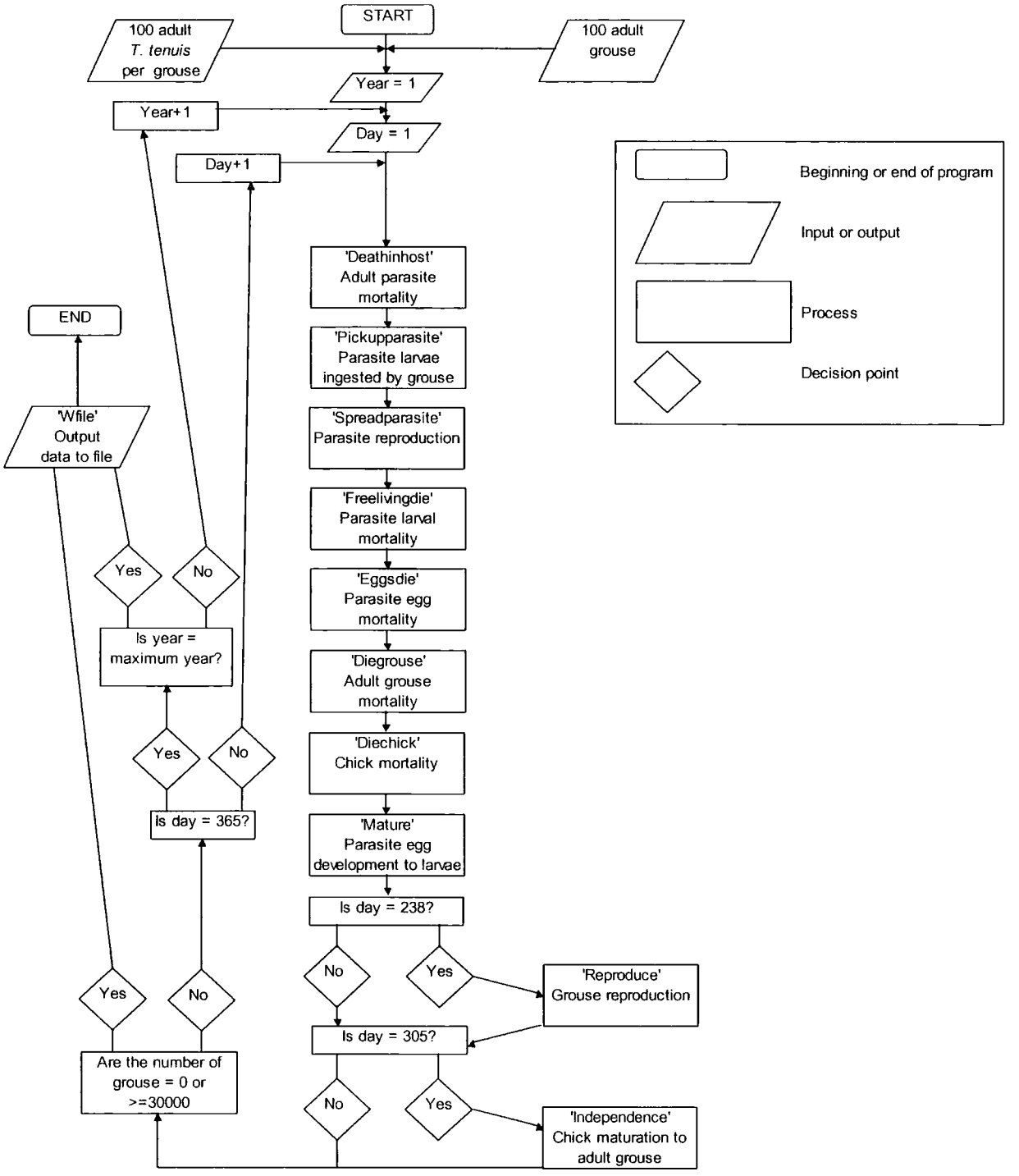


Figure 4.8 Structure of the red grouse – *T. tenuis* population model program

Table 4.4 Description of model procedures as they occur in the program

Procedure	Calculation
Deathinhost	Adult parasite mortality within the host
Pickupparasite	Parasite larvae ingested by grouse
Spreadparasite	Parasite reproduction
Freelivingdie	Parasite larval mortality
Eggsdie	Parasite egg mortality
Diegrouse	Adult grouse mortality
Diechick	Chick mortality
Mature	Parasite egg development to infective larvae
Reproduce	Grouse reproduction
Independence	Chick maturation to adult grouse

4.3.1.3 Model parameterisation

Adult parasite mortality: procedure 'deathinhost'

Adult parasites can survive for more than 2 years in captive grouse (Shaw & Moss 1989a), however extrapolation to wild grouse is difficult because captive birds tend to develop guts with smaller caecae than wild birds (Moss 1972). Grouse have little or no immunity against the parasite (Hudson 1992, Shaw and Moss 1989b) and parasite burdens increase as the host ages (Wilson 1983; Potts *et al.* 1984; Hudson *et al.* 1985; Shaw & Moss 1989b). Adult parasite survival (based on parasite egg counts) has been estimated at 34% per year in captive grouse (Hudson *et al.* 1992b) and 42% per year in wild grouse (Moss *et al.* 1993b). In the model adult parasite survival was estimated as 40 % $year^{-1}$, i.e. adult parasite mortality of 0.003 parasites day^{-1}.

Parasite transmission to the host: procedure 'pickupparasite'

T. tenuis infective larvae migrate to the growing tips of heather plants (McGladdery 1984; Watson & Hudson 1987; Saunders *et al.* 1999) which are eaten by the grouse (Hudson 1986a; Hudson *et al.* 1992). Estimation of transmission rate is difficult because the rate of host infection depends on the availability of infective larvae on the vegetation and ingestion rate. Attempts to estimate numbers of infective *T. tenuis* on vegetation have proven inconclusive (Hudson 1986a; Shaw *et al.* 1989; Saunders *et al.* 1999). In field tests the maximum mean percentage of larvae recovered from heather tips was 10.2% although this was considered an underestimate because extraction of larvae from vegetation was inefficient (Saunders *et al.* 1999).

All of the model grouse (adults and chicks) are susceptible to infection because most wild birds (at least 90%) are infected with *T. tenuis* (Hudson 1986a, Wilson 1983). Each model host ingests a proportion of parasites per day. Although larvae take approximately 12 days to develop to reproducing adults after ingestion, or delay development for several months (Shaw 1988), model larvae become adult parasites immediately. Because of the difficulty in estimating transmission accurately I used seven values for the proportion of larvae ingested: 0.000001, 0.00001, 0.0001, 0.001, 0.005, 0.01 and 0.03 larvae grouse^{-1} day^{-1}.

Parasite reproduction: procedure 'spreadparasite'

Adult parasites mate within the caecae and the females produce eggs that pass out of the birds in the caecal droppings once each day (Moss *et al.* 1993b). Although fecundity does vary with seasonally and annually (Moss *et al.* 1993b), there is no evidence of density-dependent decrease in worm fecundity in red grouse (Shaw & Moss 1989a; Moss *et al.* 1993b; Hudson & Dobson 1997; and see chapter 3). Estimates of egg production by adult worms range between 110 eggs worm^{-1} day^{-1} (Hudson & Dobson 1992b) to 155 eggs worm^{-1} day^{-1} (Shaw & Moss 1989a). I selected a model parameter for parasite fecundity (from the mean of these estimates of 132 eggs worm^{-1} day^{-1}) of 130 eggs worm^{-1}day^{-1}.

Free-living parasite larvae mortality: procedure 'freelivingdie'

Survival and development of eggs to infective third stage larvae is dependent on humidity (Shaw *et al.* 1989) and temperature (Shaw *et al.* 1989; Connan & Wise 1993; 1994). Larvae are very susceptible to desiccation (Watson 1988, Shaw *et al.* 1989); however, they appear to be able to survive typical temperatures on a Yorkshire grouse moor in the winter (Connan & Wise 1994) contrary to earlier work by Shaw *et al.* (1989). Simulation in an incubator of temperatures from September to May on a north Yorkshire grouse moor showed that daily larval mortality varied between 0.08% (17% of larvae died within 228 days) and 6% (99.9% of larvae died within 108 days) when moisture was adequate (Connan & Wise 1994). In field tests mean daily larval mortality ranged between 0.5% (tested from September to June) (Connan & Wise 1994) to 1.8% or above in summer months (Shaw *et al.* 1989). Seven values for larval mortality were chosen based on the work outlined above: 0.0005, 0.005, 0.02, 0.05, 0.1, 0.4 and 0.8

larvae day^{-1}. The minimum in this range caused 17% of larvae die in one year, the maximum caused more than 99% of larvae to die within 3 days.

Parasite egg mortality: procedure 'eggsdie'

Development of eggs to the infective larvae depends on environmental conditions (Shaw *et al.* 1989; Connan & Wise 1993; 1994) although eggs are generally less hardy than larvae. Significant development is unlikely on moorland from November to January (Connan & Wise 1993). In field tests yields of larvae varied between 54% in June and 1.0% in October and in laboratory tests the greatest yield of larvae from eggs kept at different fixed temperatures was 52% (Shaw *et al.* 1989). In later experiments, at fluctuating temperatures similar to field conditions, mortality varied between 3.6% and 1.4% per day. Daily mortality of frozen eggs was as high as 77% (Connan & Wise 1993). Six values for parasite egg mortality were chosen: 0.005, 0.02, 0.05, 0.1, 0.4 and 0.8 eggs day^{-1}. Here the minimum mortality causes 84% of eggs to die one year, and maximum mortality causes more than 99% to die within 3 days.

Adult grouse mortality: procedure 'diegrouse'

In the model, adult grouse are subject to 'natural' (all sources of mortality except parasite) and parasite induced mortality. 'Natural' mortality was estimated from work showing that the life expectancy of a grouse is 2 years (Jenkins *et al.* 1963; Hudson 1986a) (equivalent to 0.19% daily mortality). This estimate includes parasite induced mortality, therefore three values for 'natural' adult mortality were chosen: 0.0025, 0.0015, 0.0005 grouse day^{-1} (a range from 60 to 17% of grouse $year^{-1}$). Parasite-induced mortality is difficult to quantify. Grouse body condition declines with a parasite burden of more than 3000 worms (Hudson 1986a) and high burdens can result in death (Wilson & Wilson 1978; Hudson 1986a). However there is not a clear relationship between the number of adult worms in a bird and its condition (Jenkins *et al.* 1963). In the model, therefore, parasite-induced mortality is a linear function of host parasite burden (greater parasite burdens result in a greater chance of mortality). The parasite burden of individual grouse can be as high as 30000 worms per bird (Wilson 1983; Hudson & Newborn 1987); this value was therefore made the maximum parasite burden in a model host.

Chick mortality: procedure 'diechick'

Model chicks are also subject to 'natural' and parasite induced mortality. Chick mortality varies considerably over time, with high mortality in the first few days (Hudson 1986b) and the majority of mortality occurring in the first two weeks (Jenkins *et al.* 1963). Studies in Scotland showed an average of 52% of chicks survived to adults in late summer (Jenkins *et al.* 1963) and in the north of England survival to 6 weeks ranged from 84.5 to 64.6% (Hudson 1986b). These estimates include the influence of the parasite and therefore three values for 'natural' chick mortality were chosen: 0.002, 0.0085, 0.018 chicks day^{-1} (a range from 87% to 30% surviving to adulthood). The parasite therefore has an indirect effect on breeding because chick survival depends on the parent parasite burden. Model chicks are also subject to parasite induced mortality because chicks as young as 7 days old can be infected (Jenkins *et al.* 1963). Parasite induced mortality is a linear function of parasite burden as described for adult birds. In addition model chicks die if the parent bird dies.

Parasite egg development to infective larvae: procedure 'mature'

Parasite egg development to infective larvae depends on temperature and moisture (Watson 1988; Shaw *et al.* 1989; Connan & Wise 1993, 1994). In field tests egg development to infective larvae ranged from 9 days (optimum conditions) to 31 days (excluding months where eggs did not develop) (Shaw *et al.* 1989). Although *T. tenuis* eggs are unlikely to develop during winter, they can survive temperatures from February onwards and become infective approximately 3 months after being deposited (Connan & Wise 1993). Four different values of parasite egg development were tested: 0.14, 0.04, 0.02 and 0.01 eggs develop day^{-1}, (range: mean of 7, 25, 50 and 100 days to develop to infective larvae).

Adult grouse reproduction: procedure 'reproduce'

A red grouse clutch can be as large as 15 eggs (Jenkins *et al.* 1963) although the average brood size is 8 chicks (Jenkins *et al.* 1963; Hudson 1986a; Hudson 1992; Moss *et al.* 1993b). Hatching date varies between May 23rd and June 12th according to study area and year (Jenkins *et al.* 1963, Moss *et al.* 1993b). In the model all grouse produce 8 chicks on May 26th (day 238).

Chick maturation: procedure 'independence'

Most broods receive biparental care until they are at least 2 months old (Jenkins *et al.* 1963). Model chicks remain with the parent grouse until they mature to independent adults after 67 days on August 1st (day 305).

4.3.2 Model sensitivity analysis

The sensitivity of the model was assessed by testing the effect of certain parameters on the stability of the host and parasite population. All combinations of the parameters in table 4.5 were tested (a total of 10584 combinations). Because the model was stochastic each combination was replicated 10 times. In addition, a control test to determine the model's behaviour in the absence of the parasite was also run. This was done by testing the range of parameters outlined in table 4.5 with starting conditions of 100 hosts and 0 adult parasites.

Table 4.5 Model parameters tested in sensitivity analysis

Parameter	Value
Chick mortality (Proportion of chicks day^{-1})	0.002, 0.0085, 0.018
Adult grouse mortality (Proportion of adults day^{-1})	0.0005, 0.0015, 0.0025
Parasite larval mortality (Proportion of larvae day^{-1})	0.0005, 0.005, 0.02, 0.05, 0.1, 0.4, 0.8
Parasite egg mortality (Proportion of eggs day^{-1})	0.005, 0.02, 0.05, 0.1, 0.4, 0.8
Proportion of larvae ingested (Proportion of larvae $grouse^{-1}$ day^{-1})	0.000001, 0.00001, 0.0001, 0.001, 0.005, 0.01, 0.03
Parasite egg maturation to larvae (Proportion of eggs day^{-1})	0.01, 0.02, 0.04, 0.14

When each model finished, it produced an output file containing the year, day, number of hosts, number of adult and free-living parasites and the reason the model finished. A model run finished for one of three reasons: 1: the host population went extinct (all birds died); 2: the model completed the maximum time of 50 years; 3: the host population reached the maximum of 30000 individuals. The outcome of the model was of interest because populations that went extinct or reached the maximum number of individuals indicated that the host population was unstable. Those parameters that allowed host population persistence may indicate that the host population was stable (at

least for 50 years) and can be used as a starting point to look for cycles in the host population.

Sensitivity analysis described above, highlighted the effects of certain parameters on the model host population. To demonstrate the main effects, figures illustrating the parameter space tested, and the response of the host population were drawn in Sigma plot (version 7, SPSS Inc. Chicago, USA). Shaded areas indicate host population dynamics in the majority of replicates (6 or more replicates out of 10). Shaded areas were drawn automatically in Sigma plot by extrapolating from points that had been measured directly.

4.3.3 RESULTS

4.3.3.1 Dynamics of the host and parasite population

When parasites were excluded from the model, the host population grew to the maximum number of individuals (30000) within 22 years. When the parasite population was included so that it detrimentally affected host mortality, 99.1% of tests (10489 out of 10584) finished when the host population went extinct in 6 or more replicates out of 10. Of the remaining 95 tests, 42 completed 50 years in 6 or more replicates out of 10; 36 finished when the host population grew to the maximum in the majority of replicates; in 2 tests the host population grew to the maximum in half the replicates and in the other half it went extinct. In the remaining 15 tests the host population persisted for 50 years in half of the replicates while the rest went extinct. The effects of certain parameters on the model host population are summarised below. Figures illustrate changes in a few parameters while the rest remain constant.

Parasite egg and larval mortality

The host went extinct when parasite mortality was low: specifically, when eggs or larvae survived more than two years (egg or larval mortality=0.5% or less). When parasite mortality was high, (when eggs or larvae survived less than 2 weeks (egg or larval mortality=40 or 80%)) the parasite was unable to establish in numbers great enough to regulate the host population which grew to the maximum. Figure 4.9a illustrates this effect for certain parameters. In some cases larvae survived long enough to establish in the host without causing host extinction and the simulations completed 50 years. The parasite was more likely to establish in the host population when parasite eggs developed to larvae quickly (figure 4.9a to d). When eggs took 7 days to develop to larvae (figure 4.9d), the parasite always established in the host population and in most cases the host went extinct.

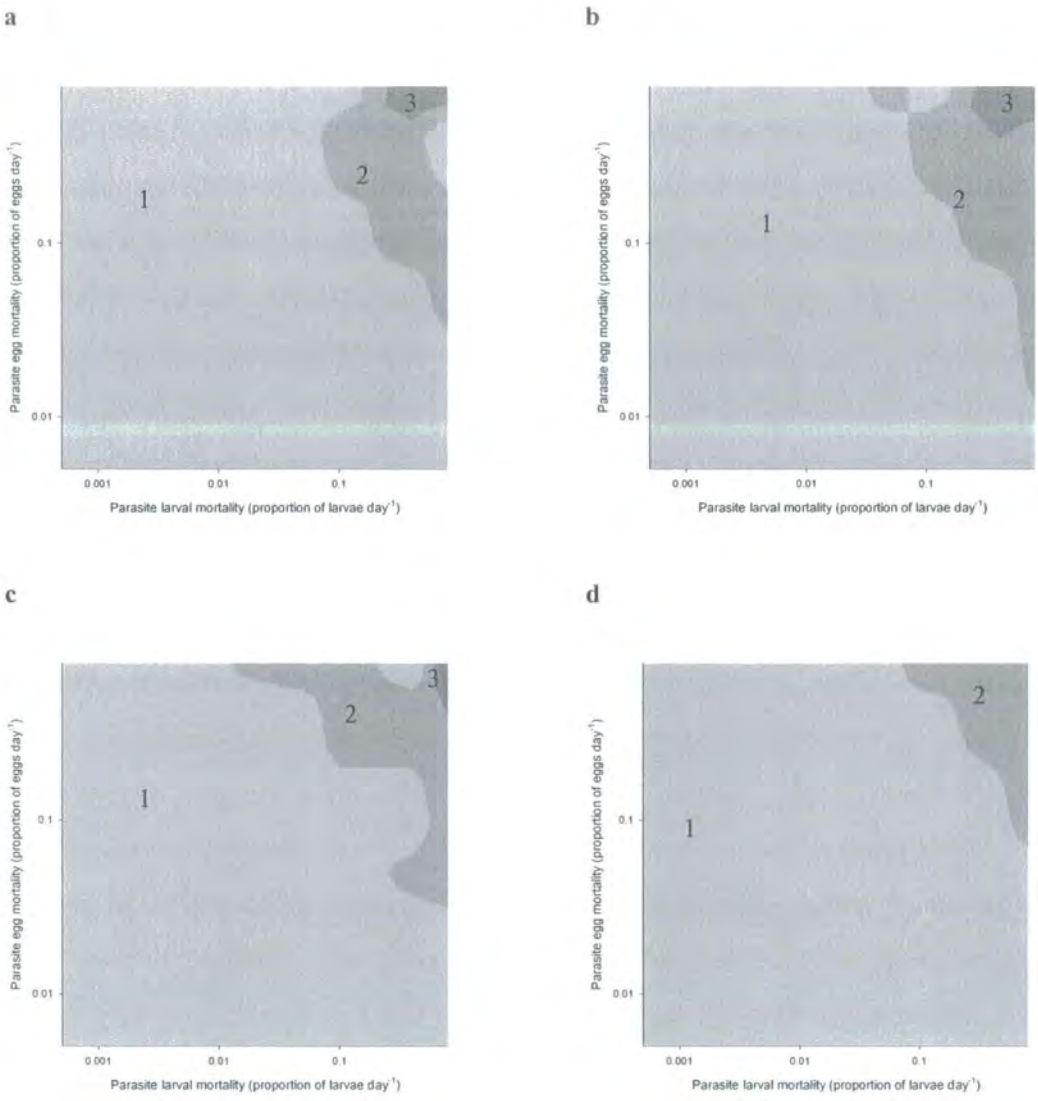


Figure 4.9 Effect of parasite egg and larval mortality on the model host population.
Shaded areas: 1: host population extinction; 2: host population persistence for 50 years; 3: host population growth to maximum of 30000 individuals. Parameter values: chick mortality=0.018; adult grouse mortality=0.0025; larval ingestion=0.000001; rate of egg development=a 0.01 (100days), b 0.02 (50 days), c 0.04 (25 days), d 0.14 (7 days).

Larval ingestion and larval mortality

The host population only persisted when the rate of ingestion was low (0.0001 larvae grouse^{-1} day^{-1} or less) and only grew to the maximum when ingestion was the lowest value tested (0.000001 larvae grouse^{-1} day^{-1}). High rates of larval ingestion (0.001 or more) caused parasite burdens to reach levels that caused host population extinction (Figure 4.10) The host population only persisted when parasite larval mortality was high and ingestion was low (figure 4.10a). When parasite egg mortality increased (b

compared to a), there was less chance of the host population going extinct because there were fewer larvae available for ingestion. Figures 4.10c and d illustrate a similar pattern when egg development to larvae was quicker (25 days). Here the parasite always had a regulatory role on the host population.

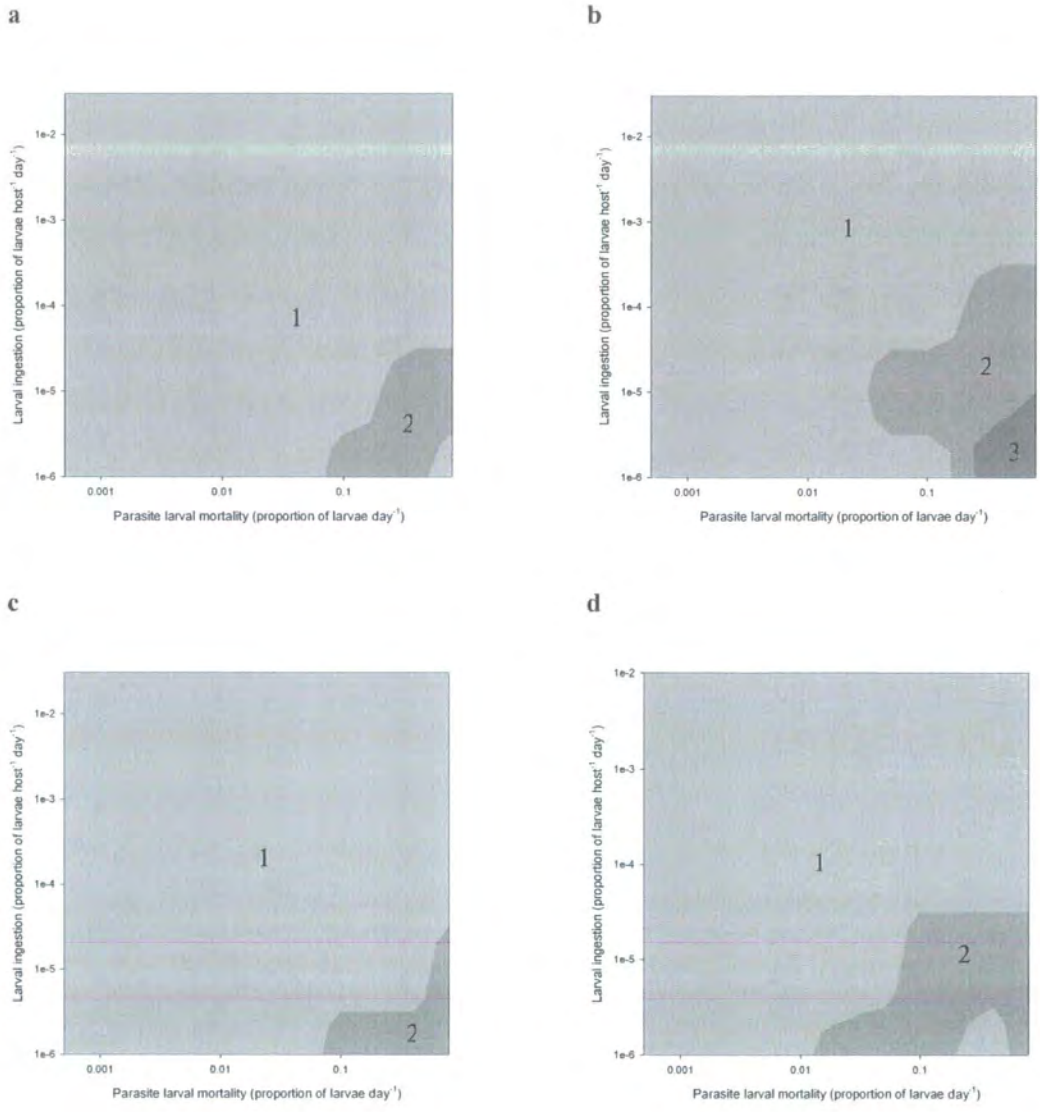


Figure 4.10 Effect of larval ingestion and larval mortality on the model host population
1: host population extinction; 2: host population persistence for 50 years; 3: host population growth to maximum of 30000 individuals. Parameter values: chick mortality=0.018; adult grouse mortality=0.0025; rate of egg development=a and b 0.01, c and d 0.04; egg mortality=a and c 0.4, b and d 0.8.

Host mortality

High rates of host mortality did not necessarily cause host population extinction. Interestingly, the host population only persisted for 50 years when chick mortality was the highest value tested (0.018 chicks day^{-1}) where only 30% of chicks survived to adulthood. Thirty six of the 42 persistent populations occurred when adult mortality was also high (0.0025 adults day^{-1}) where only 40% of the adult population survived one year. High host mortality prevented parasite burdens reaching levels that caused significant host mortality and as a result the host population grew to the maximum.

For all rates of host mortality, the parasite regulated the host population when larval mortality was low (0.02-0.1 larvae day^{-1}, i.e. larvae survived 1 to 8 months) (Figure 4.11a). When larval mortality was high (0.4 larvae day^{-1}, larvae survived less than 2 weeks) (Figure 4.11b) the parasite only established when adult and chick mortality was low. A similar pattern occurred when parasite egg development was quicker (25 days (Figure 4.11 c and d).

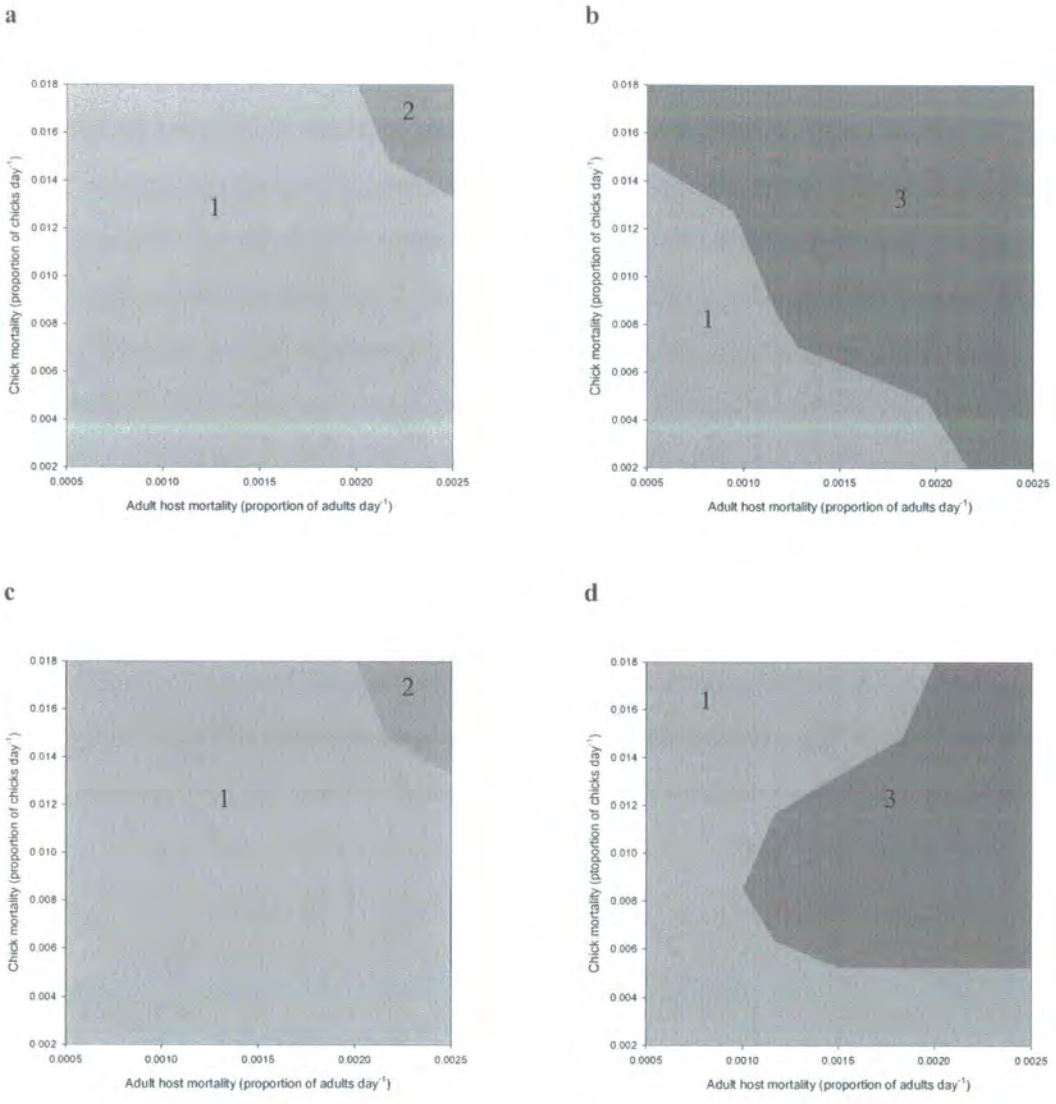


Figure 4.11 Effect of adult and chick mortality on the model host population
1: host population extinction; 2: host population persistence for 50 years; 3: host population growth to maximum of 30000 individuals. Parameter values: egg mortality=0.8, larval ingestion=0.000001; rate of egg development= a and b 0.02, c and d 0.04; larval mortality= a 0.05, b 0.4, c 0.1, d 0.8.

4.3.3.2 Host population persistence

Only 42 (of 10584, 0.4%) parameter combinations resulted in host population persistence for 50 years in 6 or more replicates out of 10. Stable parameter space is of particular interest since it may indicate either host parasite equilibrium (stable values) or population cycles. I tested the stability of this minority of parameter combinations by running each for 100 years (10 replicates each), and the host and parasite population size was output yearly. Results showed that the host population persisted for 100 years in the majority of replicates for only 4 of the 42 combinations. Further tests showed that

none persisted for 150 years in the majority of replicates. The 42 parameter combinations that persisted for 50 years occurred when chick mortality was high (0.018 chicks day^{-1}) and mostly when adult mortality was high (0.0025 adults day^{-1}). There was no clear pattern in persistence among the other parameters. Egg and larval mortality were greater than 0.05 eggs or larvae day^{-1}, while persistence occurred for all rates of egg development.

The four parameter combinations that allowed host persistence for 100 years in the majority of replicates did show cyclic type dynamics. For example, figure 4.12 shows the ten replicates of one example (one parameter combination). These time series have not been analysed for cyclicity since they are a tiny minority of the large parameter space analysed and are atypical of the model simulations.

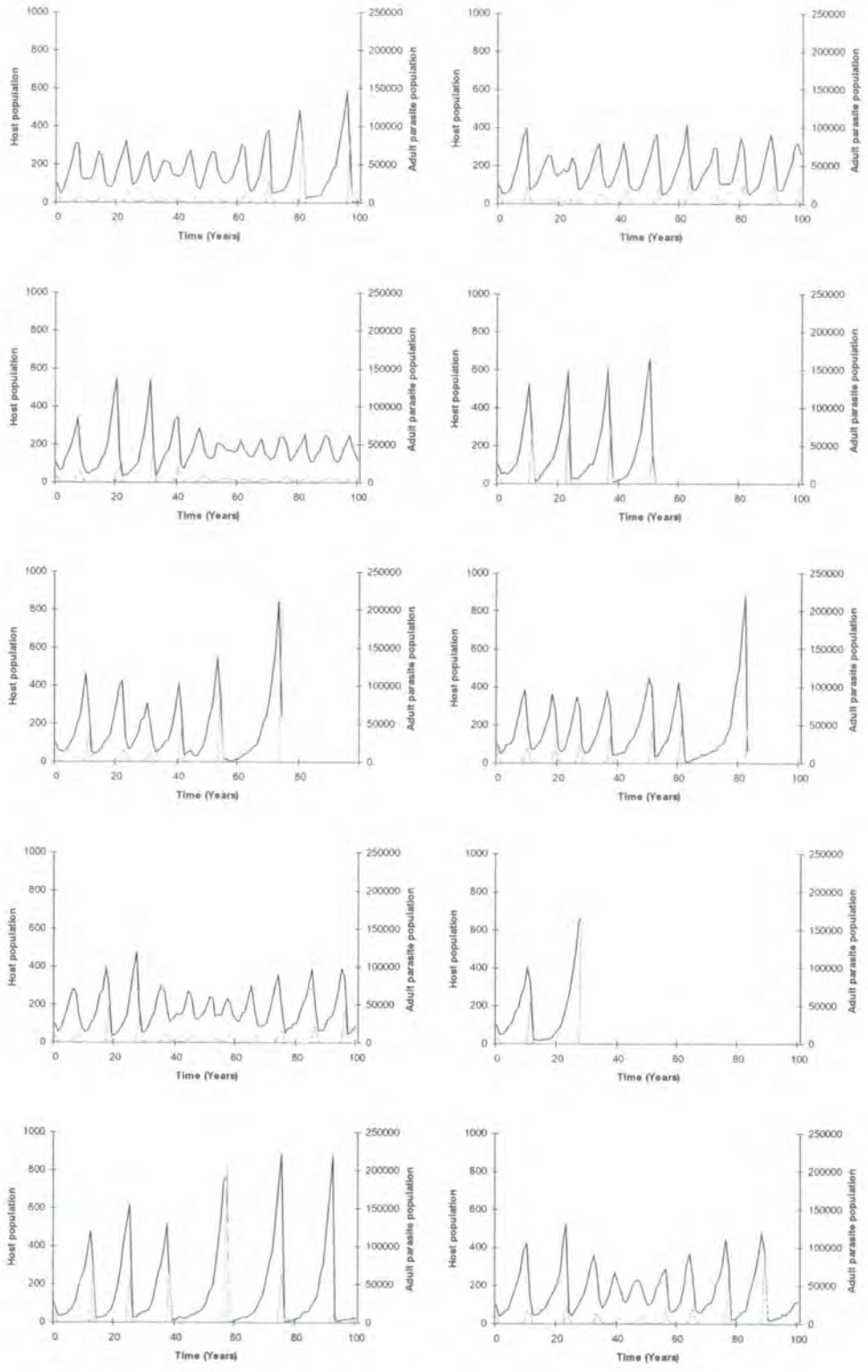


Figure 4.12 Host and parasite population abundance in 10 replicate model tests of one parameter combination. Host (black) and parasite (grey) population numbers were recorded yearly for 100 years. In three replicates the populations went extinct within 100 years. Parameter values: chick mortality=0.018; adult grouse mortality=0.0025; egg mortality=0.8; larval mortality=0.8; larval ingestion=0.0001; rate of egg development=0.01.

4.4 DISCUSSION

4.4.1 Mathematical modelling

Mathematical modelling has demonstrated that parasites can cause cycles in the abundance of their host populations through their impact on host mortality (Anderson & May 1978; May & Anderson 1978). Modified versions of these models have shown that *T. tenuis* could generate cycles in red grouse populations (Dobson & Hudson 1992). I used the mathematical models of Anderson and May to demonstrate that birth and survival of the parasite strongly influenced the occurrence and period of cycles. The tendency of the host population to cycle also increased as the survival of the free-living parasites increased. As larval life expectancy increased the period of the cycles increased. To generate cycles the free-living parasite needed to survive long enough for worm burdens to reach levels that reduced the survival or breeding of the host. Also, when the parasite caused a population crash, the infective stages needed to remain viable for long enough to reinfect the surviving hosts when numbers started to increase.

When model F was converted to discrete time, as a more realistic representation of time in red grouse populations, the host and parasite dynamics became unstable and the host population did not cycle in abundance. Discretisation of the model introduced a time lag, a factor that can cause instability in host parasite dynamics (May & Anderson 1978). The discrete model host population was unstable and either reached the maximum or went extinct. In reality for a population to persist, it would be constrained by density-dependent factors, for example, by availability of food or territories at high density. Conversely, at low density, survivors in a population may breed faster or survive longer than average; population density then returns to its usual level (Moss *et al.* 1982).

In comparison to continuous time models, I used a more realistic individual-based modelling approach, which has not previously been applied to the red grouse – *T. tenuis* system. This new approach allowed the analysis of number of factors not previously considered, and this makes it a more realistic representation of the natural system. Firstly, in the host population grouse breeding is confined to the breeding season rather than being continuous. In addition, a period of chick development to adults was

included. In terms of the parasite population, parasite egg production and egg development were included for the first time. The model also indirectly incorporated climatic factors through their impact on *T. tenuis* free-living larvae and eggs. Studies on the survival of parasite eggs and larvae (Connan & Wise 1993; 1994) describing much higher survival rates than previously thought were used to parameterise the model.

4.4.2 Individual based model

T. tenuis had a regulatory effect on the model red grouse population through its detrimental influence on host mortality, and when parasites were excluded the host population grew to the maximum. When parasites were included, the host population went extinct in 99% of simulations as a direct result of fatally high worm burdens. Conversely, in a very small number of simulations the parasite could not establish in great enough numbers to regulate the host and as a result the host population grew to the maximum. As expected this occurred when rate of parasite ingestion was low and egg and larval mortality were high. The parasite was more likely to establish in the host population if eggs developed to larvae quickly, since this kept the availability of infective stages for host infection high. When natural host mortality was low parasite burdens reached levels that caused host extinction. Surprisingly, however, high rates of natural host mortality did not cause host extinction. In fact when natural host mortality was high, parasite burdens did not have time to reach fatal levels and the host population either persisted or grew to the maximum. In support of this, when additional mortality (in the form of predation) was included in the Dobson & Hudson model of red grouse, the tendency for cycles was reduced and the equilibrium host population density increased (Hudson *et al.* 2002). Although this appears counterintuitive, the removal of a few heavily infected hosts effectively removes more parasites than grouse and so reduces the regulatory role of the parasites that cause instability (or in this model, parasite extinction).

4.4.3 Model instability

Although very few model simulations produced host population persistence over 50 years, I tested a very large range of parameter combinations, which covered and exceeded all likely life-history parameter values. Mis-parameterisation of the model is thus unlikely to explain the absence of host cycles. The model was obviously not a

complete description of the influence of the parasite on grouse populations and other important factors that allow persistence in real grouse populations may have been omitted. There are a number of factors that could stabilise populations. When the parasite was omitted from the model the host population grew to the maximum, however, in reality the growth of the population would be limited by factors such as availability of food or territories. Some density-dependent processes may stabilise the host population including density-dependent host reproduction or mortality. Dobson & Hudson (1992) included a form of territorial defence in their red grouse-parasite model so that host fecundity decreased with increasing density. This addition stabilized the host population and the parasite caused damped cycles in host abundance. Other biological processes that have a stabilizing influence on host parasite interactions include over-dispersion of parasite numbers per host, a non-linear functional relationship between parasite burden and host death rate, and density-dependent constraints on parasite population growth within individual hosts (Anderson & May 1978). All of these factors could be applied to the population model. The distribution of parasite in the host was unlikely to be over-dispersed considering each host had the same opportunity of infection. Also the parasite-induced mortality was a linear function of parasite burden. Finally there was no density-dependent constraint on parasite population growth although this aspect is unlikely to have a role in the red grouse-*T. tenuis* system since grouse have little immunity to *T. tenuis* (Shaw & Moss 1989b; Hudson 1992) and there is no evidence of density-dependent suppression of parasite egg production (Shaw & Moss 1989a; Moss *et al.* 1993b; Hudson & Dobson 1997). Modifications to the model to incorporate these stabilizing factors will be considered in the next chapter.

4.4.4 Conclusion

Parasites can play an important role in regulating the growth of wild host populations through their detrimental effects on host survival or reproduction (e.g. Gulland 1992; Albon *et al.* 2002). The individual-based model developed in this chapter showed that *T. tenuis* can regulate grouse population growth through its detrimental effects on host survival and reproduction. In general, the model produced unstable dynamics with the host population either growing to the maximum or going extinct. Only a small number of simulations showed some evidence of fluctuating dynamics over 150 years,

suggesting that density-dependent effects on host populations may have a significant role in the persistence of grouse populations and the occurrence of host cycles. This will be explored further in the subsequent chapter.

CHAPTER 5

Spatial modelling of a red grouse population and the effect of the parasite *Trichostrongylus tenuis*

ABSTRACT

I developed an individual-based spatial model by refining the model described in chapter 4, which specifically described the red grouse-*T. tenuis* interaction. The refined model showed that the parasite could theoretically cause cycles in grouse abundance, with the spatial distribution of both the host and parasite being important in the occurrence of cycles. Density-dependent host mortality had a stabilising influence on the host population, although the parasite still generated cycles in host numbers. In some cases this density dependence generated damped cycles in host numbers in the absence of the parasite. When the parasite was included it increased the cyclic tendency of the host population. The period of cycles was similar to those recorded in natural red grouse populations and was influenced by parasite related parameters such as transmission and free-living parasite mortality.

5.1 INTRODUCTION

In chapter 4 I developed an individual-based stochastic model (referred to as the 'population model') to simulate a red grouse population infected with the parasite *T. tenuis.* However sensitivity analysis indicated that the model was unstable for a wide range of parameters, and in the majority of tests the host population either grew to the maximum number of individuals or went extinct. In a small proportion of tests the host population persisted for 50 years, which might indicate that host numbers were stable or cycling. The small number of model runs in which persistence was observed raises the possibility that these were chance events, resulting from the host population failing to reach maximum or minimum values in the simulation time. In this chapter I consider two additional factors, which might be important in the stability and persistence of wild grouse populations.

5.1.1 Spatial model

The first factor limiting the population model of Chapter 4 was that it did not consider the spatial distribution of the host and the parasite. In reality the distribution and movement of animals in space can have an important influence on the establishment, spread and persistence of parasitic infections in wild host populations (Mollison & Levin 1995). Because the model did not consider spatial distribution, all individuals in the host population had equal opportunities of contact with the parasite. In real populations it is very difficult to quantify the rate of parasite transmission from one host to the next, since it depends on a variety of factors that influence development, survival and distribution of the host and parasite. However it is clear that infection of individual grouse is not uniform. In fact, adult parasite burdens in red grouse populations tend to be aggregated with a small number of hosts carrying a large proportion of the parasite population (Hudson *et al.* 1992b; see also Chapter 3). This clumped pattern, which is usually best described by a negative binomial distribution (Anderson and May 1978; Shaw & Dobson 1995), can be generated by a number of biological and physical processes, including spatial heterogeneity in host exposure to infection (Keymer & Anderson 1979). Of course, the uneven distribution of host and parasite influence this aggregation. Wild red grouse tend to gather in patches of suitable moorland (Savory 1978) and the infective parasites on vegetation are also thought to be aggregated, with

larvae concentrated around the caecal faeces where they develop (Saunders 1999). This observation was supported by my results (Chapter 3) demonstrating an aggregated distribution of eggs among caecal faeces.

5.1.2 Territorial model

The use of spatial individual-based population models has sometimes been criticised for being too complex in comparison to the quality of data available for parameterisation (Ruckelshaus *et al.* 1997; May 2004). However stochastic simulation models may be more appropriate than simple deterministic models to analyse disease spread where populations (such as host and parasite) are distributed in patches in a heterogeneous landscape and thus where disease spread is influenced by individual-based events (Rushton 2000). These approaches have been used successfully to study, for example, the spread of bovine tuberculosis in badgers (White & Harris 1995a,b); spread of rabbit haemorrhagic disease in wild rabbits (Fa *et al.* 2001) and territorial behaviour in red grouse (Hendry *et al.* 1997). The red grouse-*T. tenuis* system is also relatively free from the problem of parameterisation, since a large body of literature now exists detailing the biology of the two species.

The second source of instability in the original model relates to the unchecked growth of the grouse population. In a natural population such growth would be restricted by some form of density-dependent regulation (Sutherland 1996). One such density-dependent effect is territorial defence. Red grouse are territorial before and during the breeding season in spring with territories being established and maintained through aggressive behaviour (Jenkins *et al.* 1963). Territorial defence influences territory size, the quality of the territory occupied and the number of individuals failing to breed. Almost all birds that fail to establish a territory disappear or die before the breeding season (Watson & Jenkins 1968; Hudson 1992; Watson *et al.* 1994). Clearly territoriality is an important aspect of grouse population dynamics with consequences for distribution of the host and parasite, and for the spread of parasites. Indeed previous mathematical models of red grouse, territoriality has been incorporated as an important density-dependent factor limiting host population numbers (Dobson & Hudson 1992).

5.1.3 Aims

The population model developed in Chapter 4 was modified to incorporate two important aspects of the grouse *T. tenuis* system. Initially, I describe a modification to the population model incorporating a spatial aspect (spatial model). A second model was then developed which added territorial defence into the spatial model (territorial model). These models were used to explore which features of the host-parasite interaction might influence the occurrence of host population cycles and to compare any cycles produced to those observed in wild red grouse populations.

The methods and results are divided into two sections. Section 1 describes the design and parameterisation of the model and the sensitivity analysis. Using sensitivity analysis, I identify the parameter space where the host population was unstable (reaching the maximum or going extinct), and, more importantly, the parameter combinations that allowed host population persistence. Persistence can then be used as a starting point to look for cycles in host population numbers. I continue by focusing on the parameter space where the host was persistent, to look for cycles in host population numbers. I describe this further analysis in the methods and results of section 2.

SECTION 1 – MODEL DESIGN AND SENSITIVITY ANALYSIS

5.2 METHODS

5.2.1 Model structure

A spatial model of red grouse and the parasite *T. tenuis* was constructed by expanding the population model developed in chapter 4. The spatial population model (like the population model) simulates life histories of individual red grouse in a population and the associated *T. tenuis* parasite population. The main events in the host and parasite life cycle incorporated in the model are the same as described in section 4.3.1 and illustrated in figure 4.7 (chapter 4). A second model was also considered in which the spatial model was modified to include the effect of territoriality (the territorial model).

A simple method of incorporating spatiality into a model is to represent space as a regular array of sites (lattice or grid models), with one individual or population sub-group at each. The population model was modified to include a spatial component by forming a 50 x 50 cell grid. For simplicity the grid was two dimensional and spatially homogenous. Realistic parameter values, which are further described below, were estimated from the literature. Each grouse was allowed to occupy any position on the grid, and more than one grouse could occupy the same cell at the same time. Grouse could move randomly among adjacent cells, although immigration and emigration were excluded. The parasite was spread by the hosts depositing eggs and ingesting parasite larvae in the cells they occupied.

5.2.2 Program structure

The program was written in Visual Basic (version 6, Microsoft Corporation, Seattle, Washington, USA) (Appendix 2). The structure of the spatial program and the territorial program are illustrated by flow diagrams in Figure 5.1. The simulation begins on day one with 100 adult grouse, 100 adult parasites per grouse, 0 free-living parasite larvae and 0 parasite eggs. Adult grouse are distributed randomly on the grid at the start of the program. The program executes a consecutive set of procedures (table 5.1) that calculate changes in the host and parasite population each model day using the population size from the previous day. As well as including a number of additional parameters to

describe the spatial distribution and territoriality components of the new models, it was necessary to modify existing procedures in the population model. A small number of procedures were unchanged between all models. These additions and modifications are listed below. Detailed information about the choice of parameter values in the procedures can be found in chapter 4.

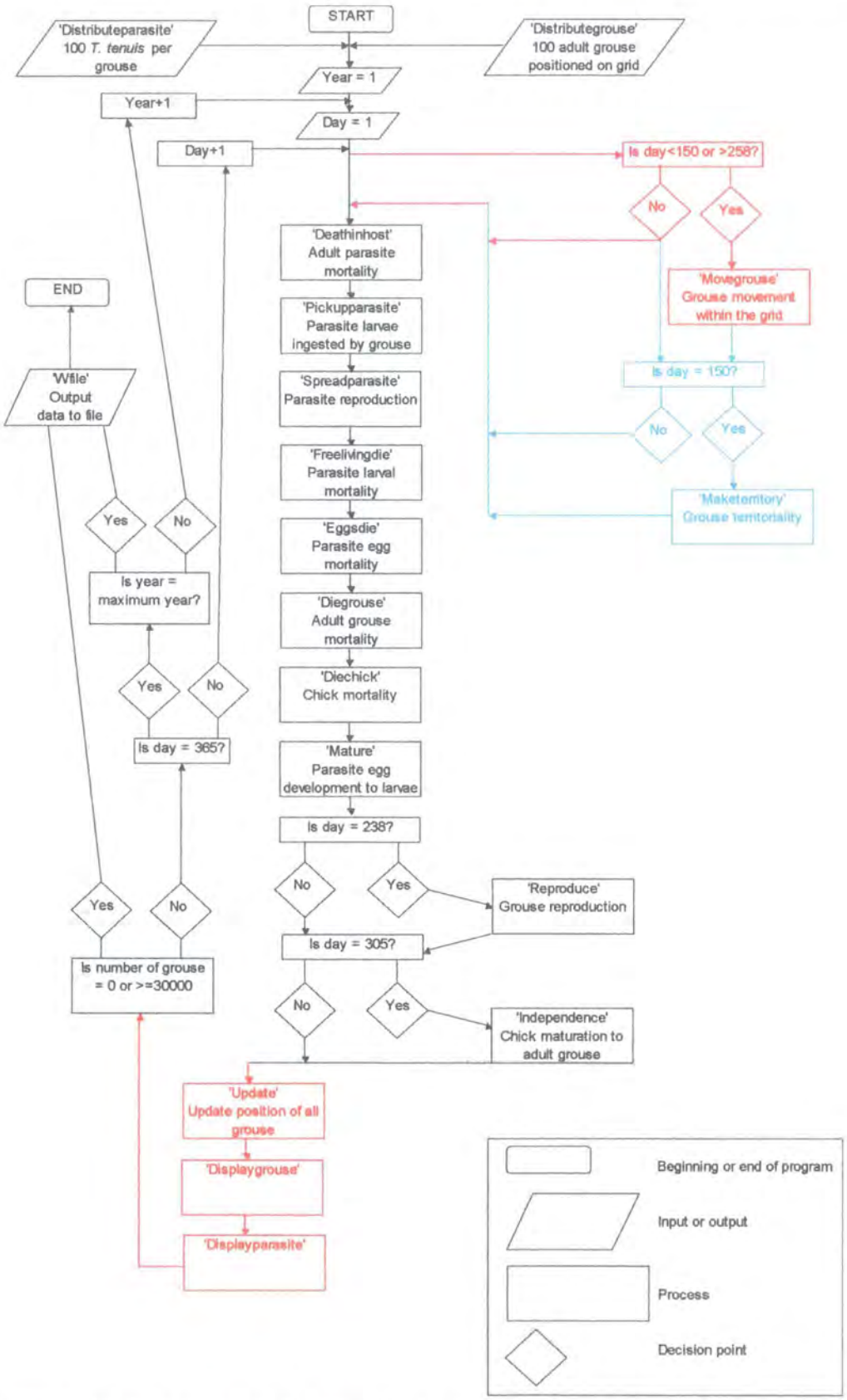


Figure 5.1 Flow diagram of the spatial and territorial population model program. The original population model is shown in black. Additions to form the spatial model are shown in red; further additions to form the territorial model are in blue.

Table 5.1 Description of spatial model procedures as they occur in the model program

Procedure	Calculation
Distributegrouse*	Grouse distributed randomly on the grid at the start of the program
Distributeadultparasite*	Each grouse infected with adult parasites at the start of the program
Movegrouse	Grouse movement between cells on the grid
Maketerritory#	Grouse form territories, other grouse in that territory die
Deathinhost	Adult parasite mortality within the host
Pickupparasite	Parasite larvae ingested by grouse
Spreadparasite	Parasite reproduction – parasite eggs are deposited in the cell occupied by the individual grouse
Freelivingdie	Parasite larval mortality
Eggsdie	Parasite egg mortality
Diegrouse	Adult grouse mortality
Diechick	Chick mortality
Mature	Parasite egg development to infective larvae
Reproduce	Grouse reproduction
Independence	Chick maturation to adult grouse

*Procedure only executed at the start of the program to set initial conditions
Procedure only occurred in the territorial model

5.2.2.1 New procedures

Initial host conditions: procedure 'distributegrouse'

At the start of the program 100 grouse were distributed randomly on the grid.

Initial adult parasite conditions: procedure 'distributeparasite'

Each grouse was infected with 100 adult parasites.

Grouse movement around the grid: procedure 'movegrouse'

Individual model grouse can move a maximum of one square per day. The probability that an individual moves on any given day is 0.5. This is an arbitrary value, as there is no indication in the literature about how often grouse move a significant distance. In general however, grouse are sedentary (Jenkins *et al.* 1963). In a study where 793 ringed juvenile grouse were recovered, 84% were recovered within 1.5km of the ringing location and 94% within 5km.

Direction of movement is random with individuals being able to move to any of the eight neighbouring cells, although individuals can not move out of the grid. A random walk may be a very coarse approximation to wild grouse movement, which will also be influenced by a variety of extrinsic factors including time or spatial constraints; however it is the simplest starting assumption when examining the effect of movement

patterns on population dynamics. Figure 5.2 shows the trail of one individual model host following a random walk. Individuals stop moving and occupy the cell that they are in on February 17th (day 150) and remain there until June 15th (day 258). This time represents the territorial and breeding season of wild grouse. The two models (spatial and territorial) differ at this point: in the spatial model, more than one grouse can occupy the same cell at any time while in the territorial model no more than one grouse can occupy each cell while hosts are sedentary (see 'maketerritory' procedure below).

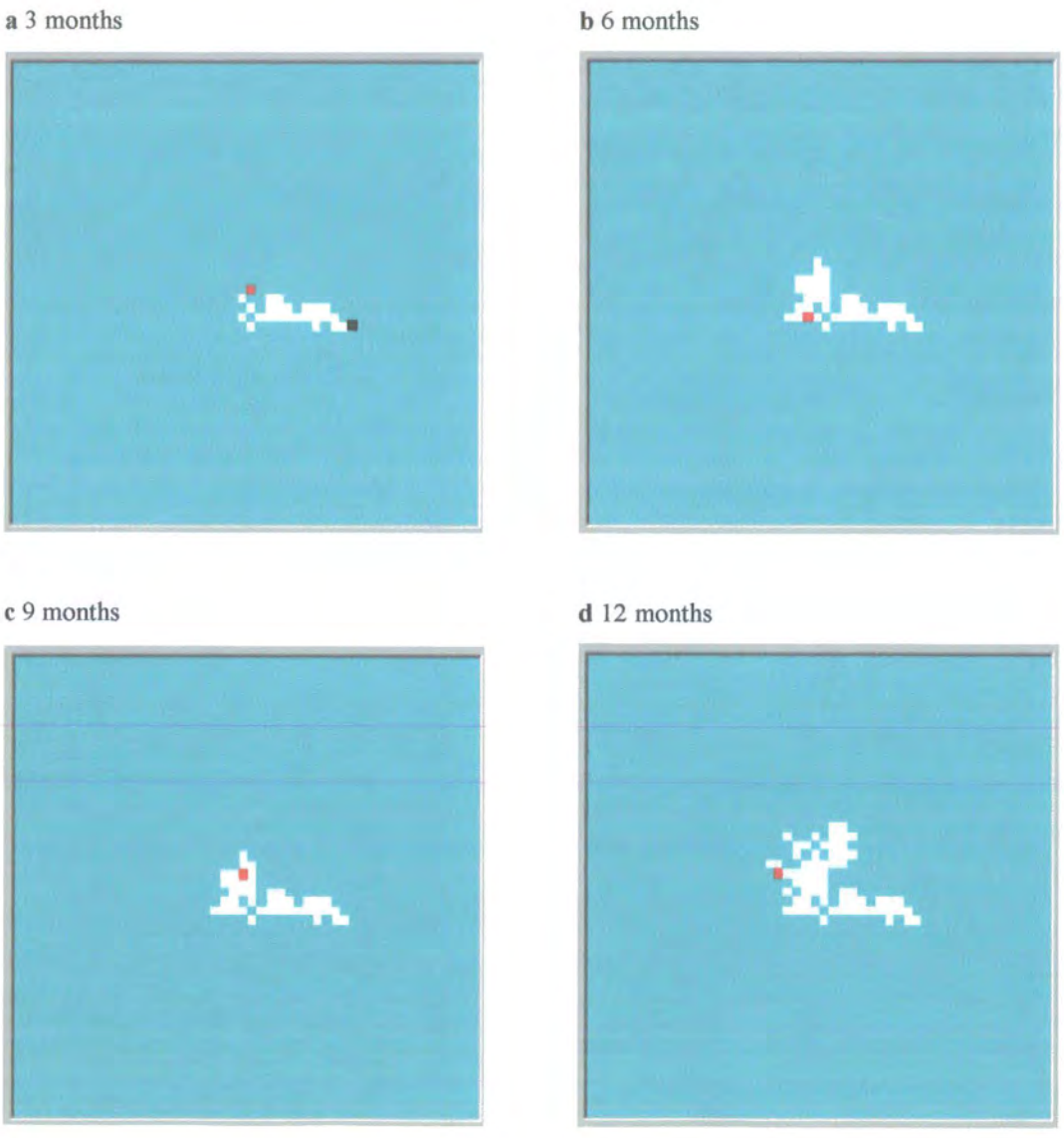


Figure 5.2 Random walk of one individual host in the spatial model
Host (red), start cell on day one (black) and the trail of its movement (white) over one model year.

Adult grouse territorial defence: procedure 'maketerritory'

In the territorial model I included a simple representation of territorial aggression. Wild red grouse tend to be territorial from February through to about June (Jenkins *et al.* 1963). Some territorial aggression occurs from November to January, although territories are abandoned in bad weather (Jenkins *et al.* 1963). Territory size fluctuates according to resources (Moss *et al.* 1988) and the amount of aggression between neighbours (Jenkins *et al.* 1963; Watson 1984). Territorial defence can therefore result in strong density-dependent mortality through individuals failing to obtain territories, since almost all non-territorial birds die or disappear (Watson & Jenkins 1968; Watson *et al.* 1994).

Territories can be as small as 1 hectare when grouse density is high (50 pairs of grouse km^{-2}) up to sizes of 4 hectares (2.5 pairs km^{-2}) (Hudson & Watson 1985; Moss & Hudson 1990), with a typical intermediate density territory size of approximately 3 hectares (30000m^2) (Hendry *et al.* 1997). In the model population the minimum size of a host territory is 3x3 cells when host density is high. Comparison to a wild population where the minimum territory (at high density) is 1 hectare allows estimation of the scale of one cell to be approximately 1089m^2 (33m x 33m). This concurs with the typical movement of individual hosts where the farthest a grouse could move in a year (given a 0.5 chance of moving per day and assuming all movement in one direction) would be 129 cells, i.e. 4.3km. The total grid size is approximately 2.72km^2 (1650m x 1650m).

For simplicity territory size was uniform and individuals formed territories with at least 1 cell distance to the next individual. The procedure selected a bird at random, it specified the cell where it was positioned on day 150 (February 27th) as its territory, and then removed any other grouse within one cell of that bird. Non-territorial birds therefore effectively died. The procedure continued to randomly select birds and specify territories until all birds had either formed a territory or had been removed.

Graphical representation: procedure' displayparasite' and procedure 'displaygrouse'

Procedures in the program: 'displayparasite' and 'displaygrouse' provide a visual representation of the grid, the host and the parasite populations.

5.2.2.2 Altered procedures

Parasite transmission to the host: procedure 'pickupparasite'

Each host ingests a proportion of the free-living parasites that are present in the cell that the host occupies. A range of values for parasite ingestion were tested (Table 5.2).

Parasite reproduction: procedure 'spreadparasite'

Adult parasites within each host produce eggs every day, which pass from the host to the cell of the grid that the host occupies. Rate of parasite egg production remains the same as in the population model.

Free-living parasite larval mortality: procedure 'freelivingdie'

Larval mortality remains the same as in the population model. I introduced a limit on the minimum number of larvae in each cell so that if there were 10 or less larvae then all larvae went extinct in that cell. This limit was required to prevent larval numbers decreasing to extremely low levels without reaching zero in any cell.

Parasite egg mortality: procedure 'eggsdie'

The probability of egg mortality remained the same as in the population model. If the number of eggs in a cell was 10 or less then the eggs in that cell all went extinct for the same reasons described above for larval mortality.

Parasite egg development to infective larvae: procedure 'mature'

Parasite eggs in one cell mature to free living larvae in that cell at a rate that varies between an average of 10 days and 100 days (as in the population model).

Adult grouse reproduction: procedure 'reproduce'

Adult grouse reproduce on one day per year, each producing 8 chicks. Chicks remain with the adult grouse and occupy the same cell as the parent bird until they become independent adult grouse.

Chick maturation: procedure 'independence'

Chicks mature to independent adult birds on August 1st. When chicks become independent they disperse in a random manner governed by the procedure 'movegrouse'.

5.2.2.3 Unmodified procedures

Adult parasite mortality: procedure 'deathinhost'

Adult parasites died at a fixed rate of 0.003 adults day^{-1}, equivalent to a proportion of 0.6 of the adult parasites dieing per year (as described in the population model).

Adult grouse mortality: procedure 'diegrouse'

Adult grouse were subject to 'natural' and parasite induced mortality as in the population model. The parameter 'probadnatural' represented the proportion of adults dieing per day. Parasite induced mortality was a linear relationship with the probability of mortality increasing with parasite burden.

Chick mortality: procedure 'diechick'

Grouse chicks were subject to 'natural' and parasite induced mortality as in the population model. The parameter 'probcd' represented the proportion of chicks dieing per day. Parasite induced mortality was also a linear relationship with parasite burden as described for adult grouse mortality.

5.2.2.4 Model timing

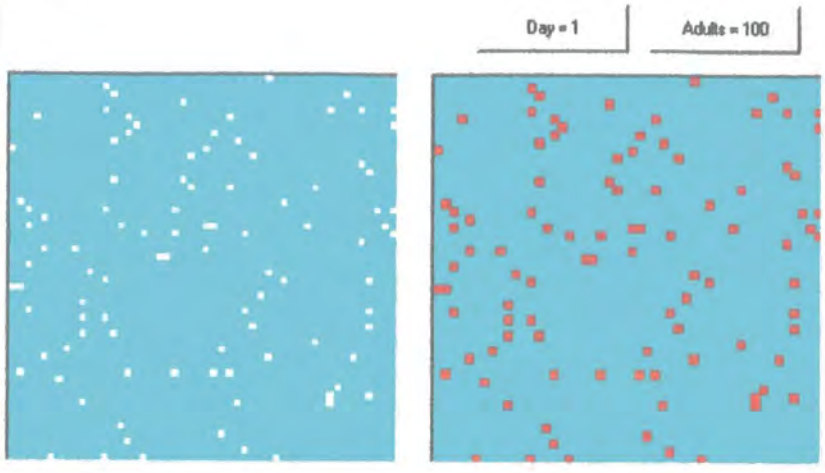
Table 5.2 shows the timing of events that occur in the spatial and territorial model in relation to both real time and model day. Figure 5.3 and 5.4 illustrate the host and parasite population numbers and distribution at certain times over one year in the spatial and territorial model respectively.

Table 5.2 Timing of events in the spatial and territorial model

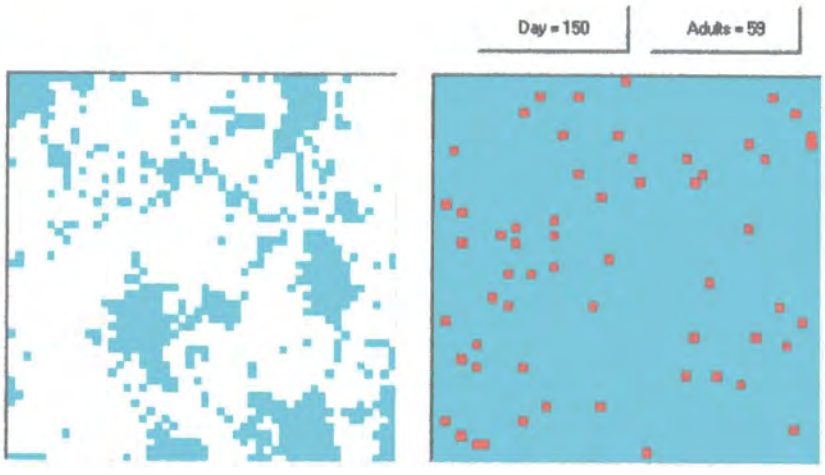
Model day per year	Action	Date
1	Start program	October 1st
150	Grouse stop moving	February 27th
150~	Grouse become territorial	February 27th
238	Grouse reproduce	May 26th
258	Grouse start moving	June 15th
305	Chicks become independent adult grouse	August 1st

~ Only in the territorial model

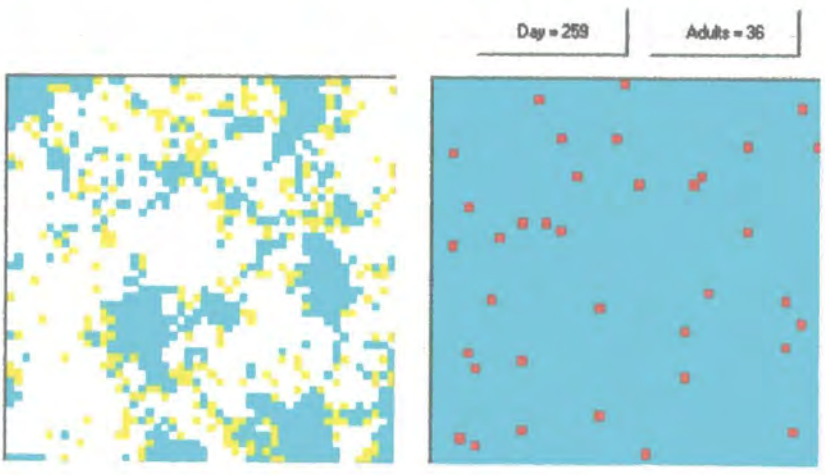
a Start



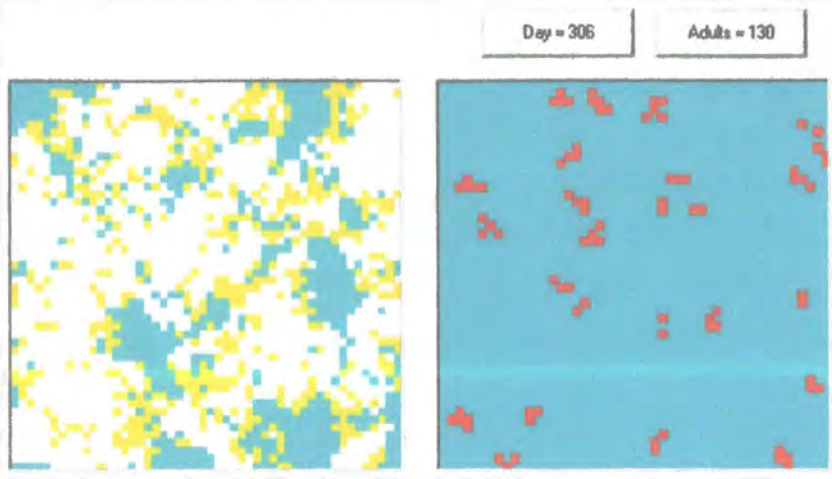
b Hosts stop moving from day 150 until day 258 (territoriality)



c Hosts start moving



d Chicks become independent adults and start to disperse



e End of 1 year

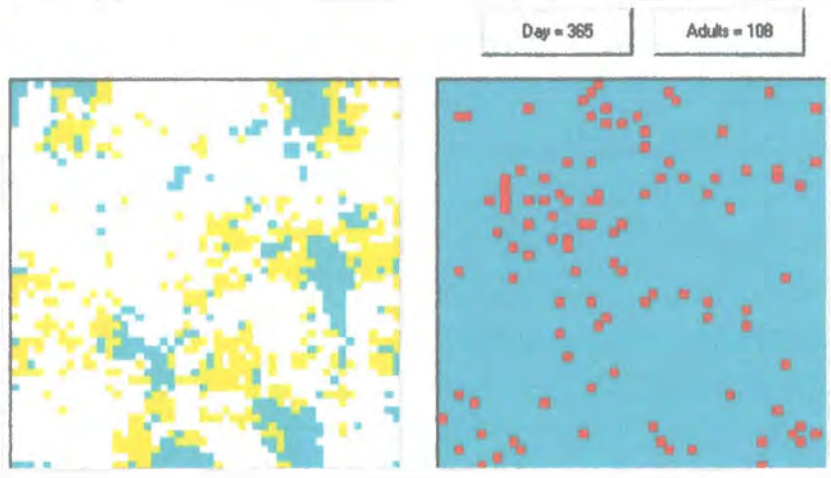
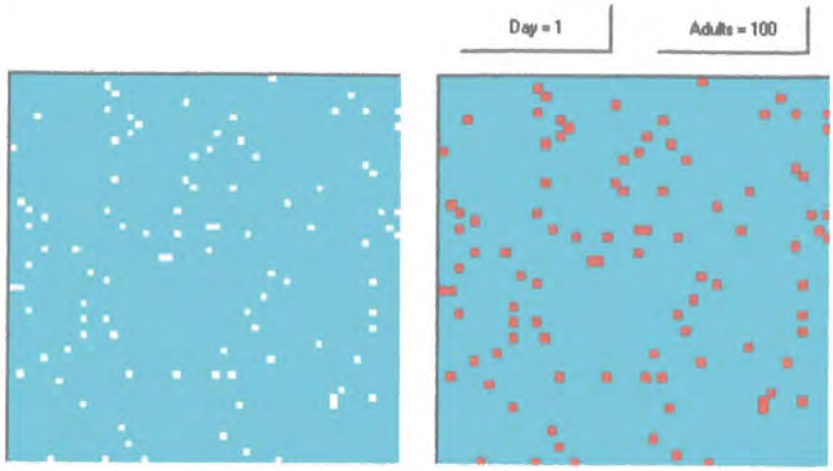
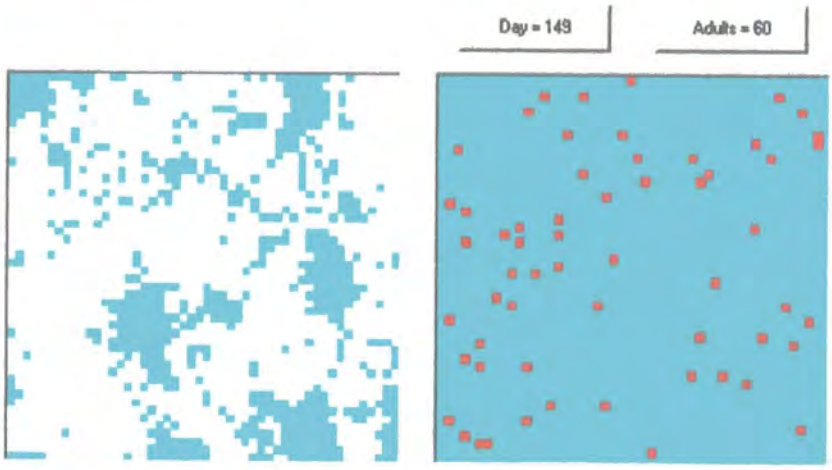


Figure 5.3 Graphical representation of host and parasite population during one year in the spatial model. Each screen capture shows the same grid of 50x50 cells. In each figure the square on the right shows the distribution of the hosts (red) on the grid (blue). The square on the left shows the cells in which there are parasite larvae (white) or parasite eggs but no larvae (yellow) on the grid (blue).

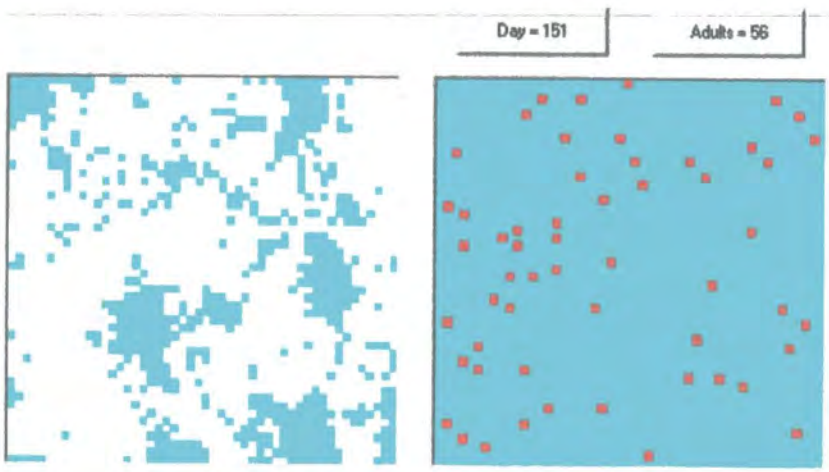
a Start



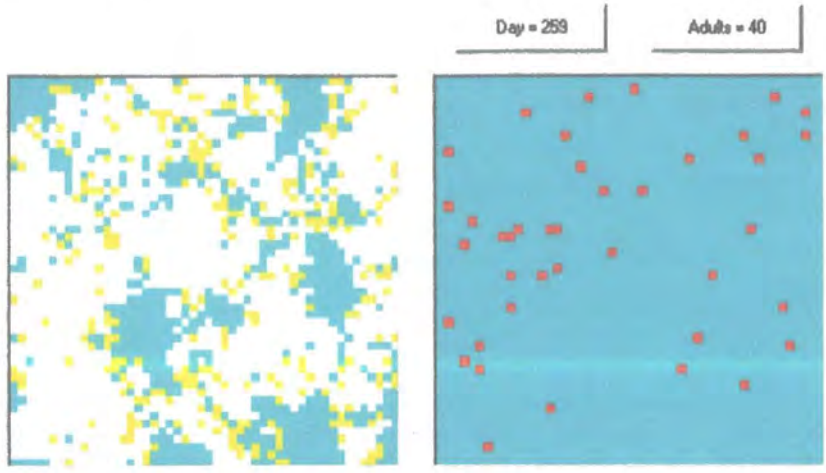
b Before territoriality



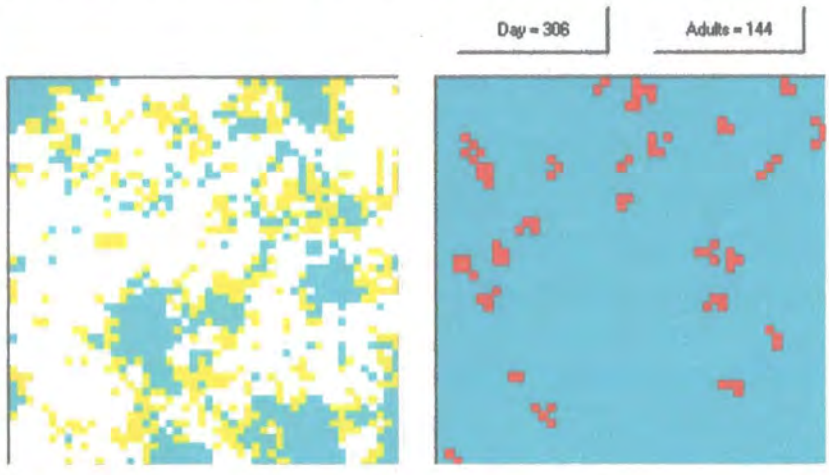
c Hosts stop moving and become territorial. Non-territorial hosts have been removed



d Hosts start moving



e Chicks become independent adults and start to disperse



f End of 1 year

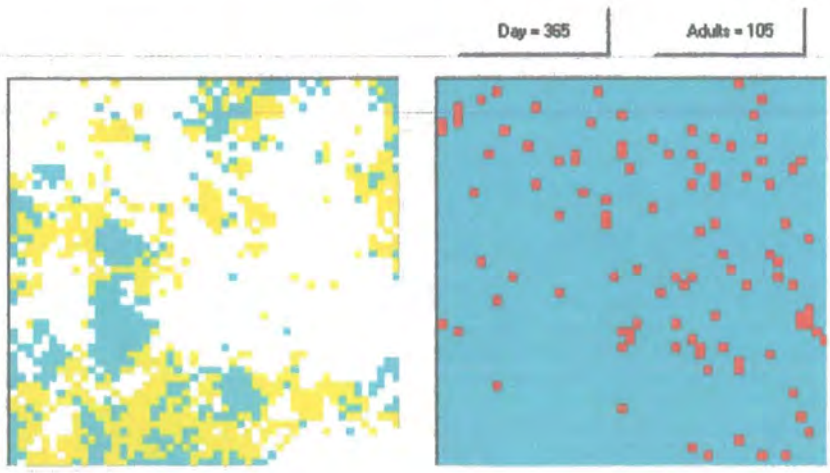


Figure 5.4 Graphical representation of host and parasite population during one year in the territorial model. Each diagram shows the same grid of 50x50 cells. In each figure the square on the right shows the distribution of the hosts (red) on the grid (blue). The square on the left shows the cells in which there are parasite larvae (white) or parasite eggs but no larvae (yellow) on the grid (blue).

5.2.3 Model sensitivity analysis

The sensitivity of certain model parameters on the stability and dynamics of the host and parasite populations was assessed by running simulations where all combinations of the parameters (summarised in table 5.1) were tested. This represented a total of 4536 parameter combinations, using the same parameters as those tested in the sensitivity analysis of the population model (the choice of values tested for each parameter is described in detail in chapter 4, section 4.3.1.3). The number of values tested was reduced (although the range of values was not reduced) because the run time for this more complicated model increased considerably over that of the population model. Because the model was stochastic, I replicated each parameter combination 10 times. In addition, a control test was run to determine the model behaviour in the absence of the parasite (starting conditions: 100 hosts, 0 parasites).

Table 5.3 Model parameters values tested in sensitivity analysis

Parameter	Value
Chick mortality (Proportion of chicks day^{-1})	0.002, 0.0085, 0.018
Adult grouse mortality (Proportion of adults day^{-1})	0.0005, 0.0015, 0.0025
Parasite larval mortality (Proportion of larvae day^{-1})	0.0005, 0.005, 0.02, 0.05, 0.1, 0.4, 0.8
Parasite egg mortality (Proportion of eggs day^{-1})	0.005, 0.02, 0.05, 0.1, 0.4, 0.8
Proportion of larvae ingested (Proportion of larvae $grouse^{-1}$ day^{-1})	0.00001, 0.0001, 0.001, 0.01
Parasite egg maturation to larvae (Proportion of eggs day^{-1})	0.01, 0.028, 0.14

When each model finished, the year, day, number of hosts, number of adult and free-living parasites and the reason the model finished were saved to an output file. A model run finished for one of three reasons: 1: the host population went extinct; 2: the model completed 50 years; 3: the host population reached the maximum of 30000 individuals. As in the population model, this allowed me to identify parameter combinations leading to population stability (i.e. model tests that ran for the specified maximum of 50 years), which can then be used as a starting point to look for cycles in population numbers.

To illustrate the main effects of parameters on the host population shaded contour figures were drawn using Sigma plot (version 7, SPSS Inc. Chicago, USA). The figures show changes in key parameters with other held constant. Shaded areas were drawn automatically in Sigma plot by extrapolating those points that had been measured directly.

5.3 RESULTS

5.3.1 Dynamics of the host and parasite population

In the spatial model, in 6% (303) of parameter combinations the host population persisted for the maximum of 50 years in 6 or more replicates out of 10. 21% (936) of tests finished when the host population grew to the maximum value of 30000 individuals in 6 or more replicates. The remaining 73% (3297) of tests ended when the host population went extinct in 5 or more replicate tests out of 10. (The criterion of 5 out of 10 was used here for the remaining tests to include those where half of the replicates ended due to extinction and half due to another cause).

In the territorial model 31% (1427) completed 50 years in 6 or more replicates out of 10. This 31% can be divided into those in which both the host and parasite persisted (7%, 337), and those in which the host populations persisted (stabilised at the carrying capacity due to density-dependent territoriality), while the parasite population went extinct (24%, 1090). The remaining 69% (3109) of runs finished when the host population went extinct in 5 or more replicate tests out of 10.

The control test (when the parasite was absent) revealed that in both models, the parasite played an important regulatory role on the host population. In the spatial model, the host population always grew to the maximum of 30000 individuals. In the territorial model, the host population always persisted for 50 years, but did not reach the maximum due to the density-dependent effects of territoriality.

Parasite egg and larval mortality

When larval mortality was low, host parasite burdens grew to levels that caused host population extinction (Figure 5.5a). When larval and egg mortality were high the host population grew to the maximum because the parasite did not reach levels that strongly influenced host population growth. In some cases the host population persisted for 50 years. Fast egg development resulted in readily available parasite larvae and therefore compensated for high free-living parasite mortality since host extinction occurred even when parasite egg and larval mortality was high. (Figure 5.5a-b). Figure 5.6a-b

illustrates exactly the same parameter space as figure 5.6 for the territorial model, and illustrates that the same pattern occurs in both models.

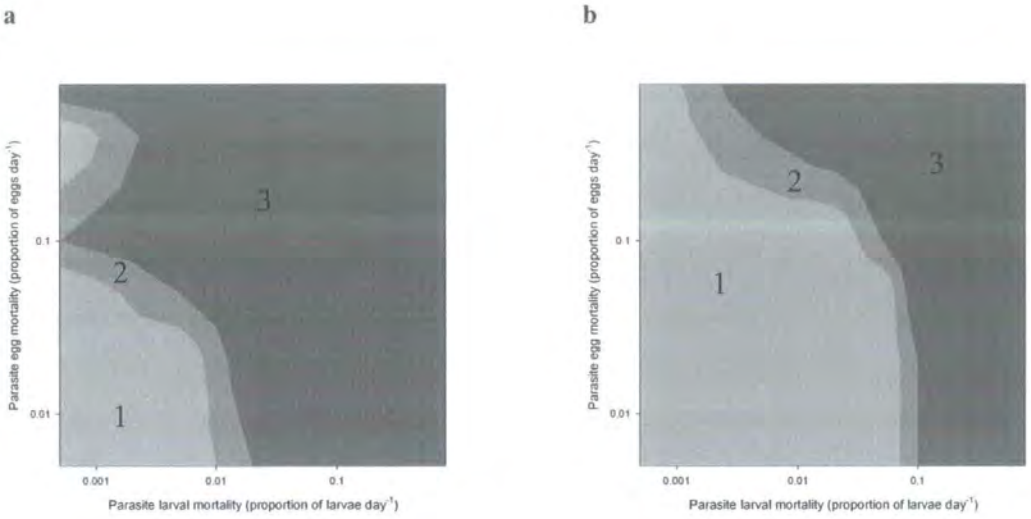


Figure 5.5 Effect of parasite egg and larval mortality on the host population in the spatial model Shaded areas indicate host population dynamics in the majority of replicates: 1: host population extinction; 2: host population persistence for 50 years; 3: host population growth to maximum of 30000 individuals. Parameter values: chick mortality=0.018; adult grouse mortality=0.0025; larval ingestion=0.00001; rate of egg development= a 0.01 (100days), b 0.14 (7 days).

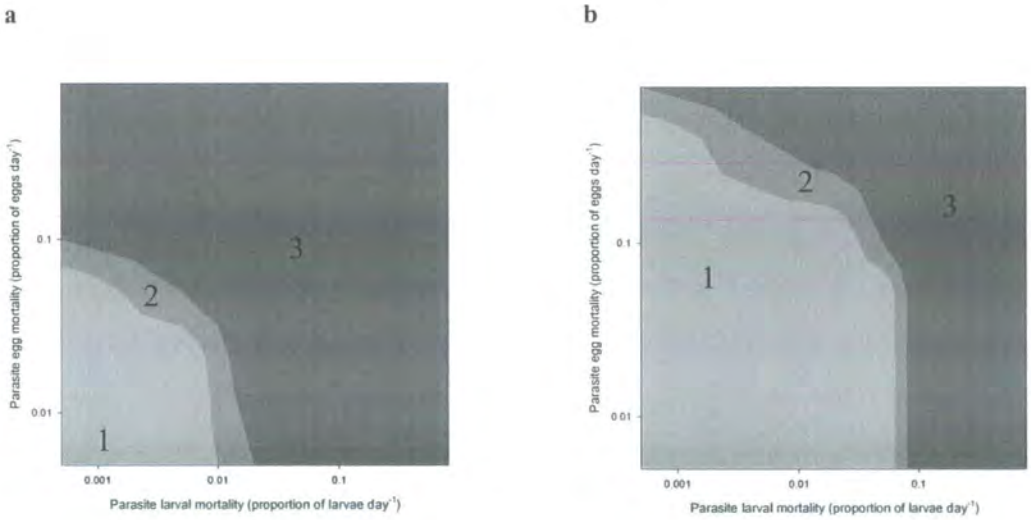


Figure 5.6 Effect of parasite egg and larval mortality on the host population in the territorial model Shaded areas indicate host population dynamics in the majority of replicates 1: host population extinction; 2: host population persistence for 50 years; 3: host population persistence and parasite population extinction. Parameter values: chick mortality=0.018; adult grouse mortality=0.0025; larval ingestion=0.00001; rate of egg development= a 0.01 (100days), b 0.14 (7 days).

Larval ingestion and larval mortality

The host population went extinct when larval ingestion was high and larval mortality was low. The host persisted or grew to the maximum when ingestion decreased and larval mortality increased (figure 5.7a). When parasite egg mortality increased, the host population grew to the maximum in the same parameter space (figure b compared to a). Faster egg development (figures c and d -7 days development, compared to a and b – 100 days development) resulted in host extinction more often. A similar pattern occurred in the territorial model (Figure 5.8).

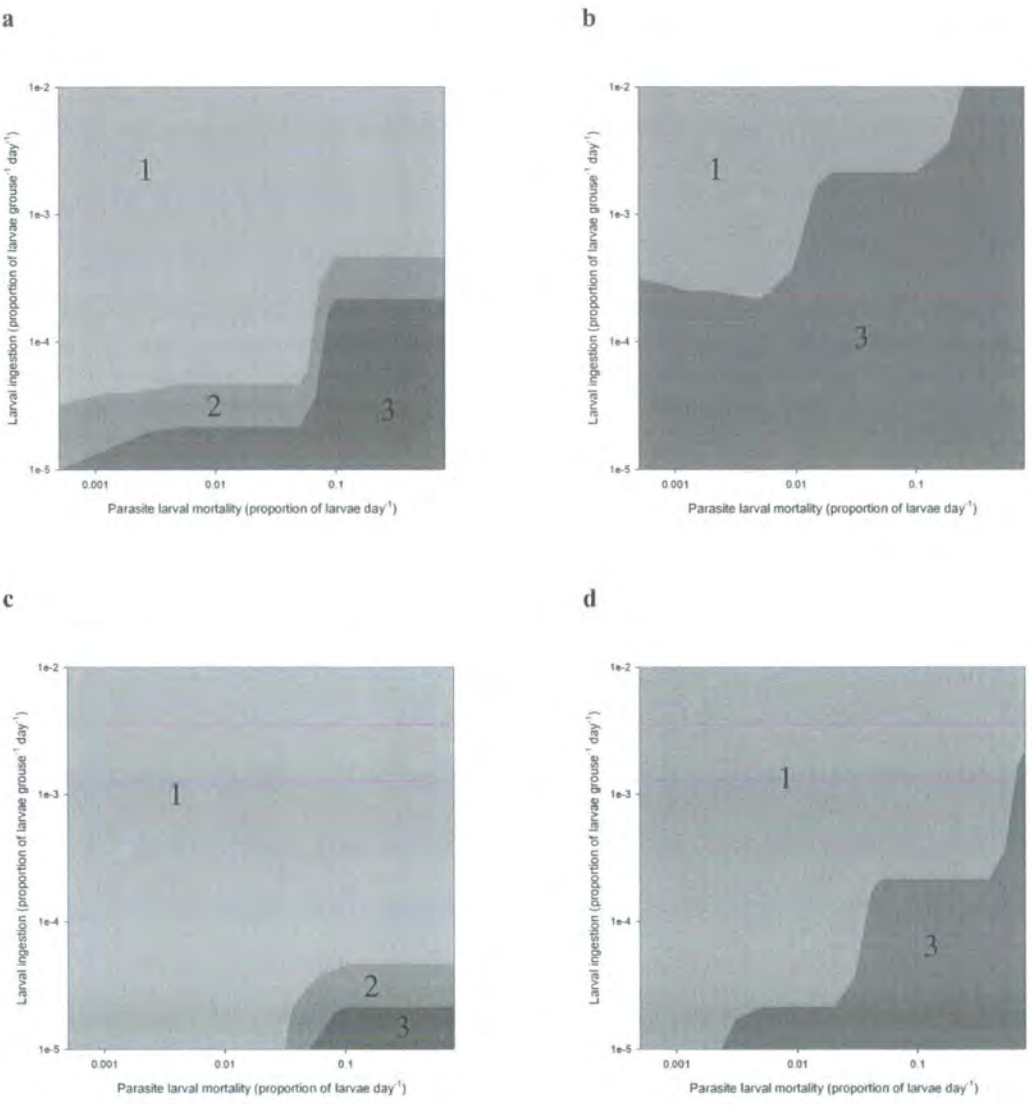


Figure 5.7 Effect of larval ingestion and larval mortality on the host population in the spatial model Shaded areas indicate host population dynamics in the majority of replicates 1: host population extinction; 2: host population persistence; 3: host population growth to maximum of 30000 individuals. Parameter values: chick mortality=0.018; adult grouse mortality=0.0025; rate of egg development=a and b 0.01; c and d 0.14; egg mortality=a and c 0.1; b and d 0.8.

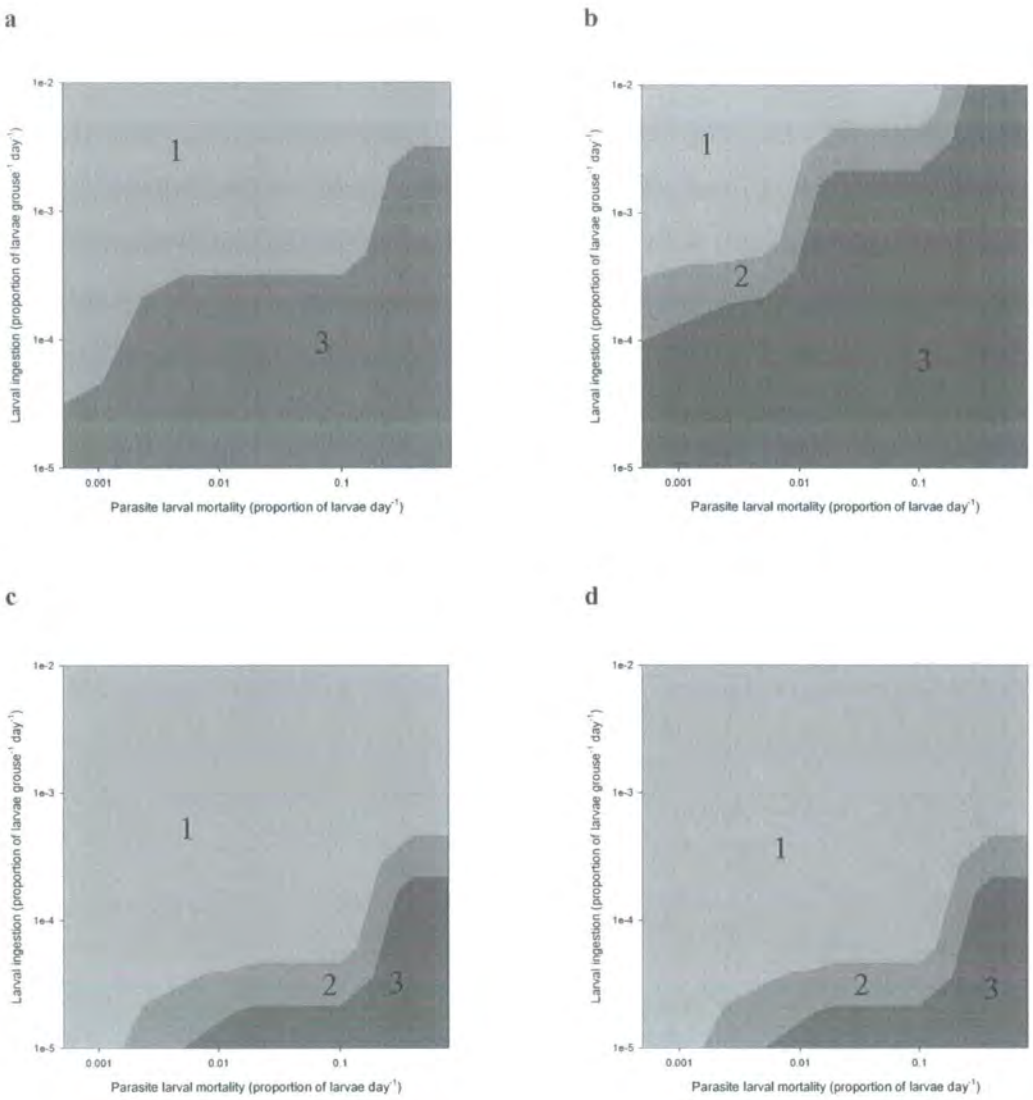


Figure 5.8 Effect of larval ingestion and larval mortality on the host population in the territorial model. Shaded areas indicate host population dynamics in the majority of replicates 1: host population extinction; 2: host population persistence; 3: host population persistence and parasite population extinction. Parameter values: chick mortality=0.018; adult grouse mortality=0.0025; rate of egg development =a and b 0.01; c and d 0.14; egg mortality=a and c 0.1; b and d 0.8.

Host mortality

Host dynamics depended on the combined influence of 'natural' and parasite-induced mortality. High 'natural' mortality did not necessarily cause host extinction. For the values illustrated in figure 5.9, the host population went extinct when larval mortality was low, while intermediate levels of larval mortality allowed host persistence in most cases (Figure 5.9a and b). The host population only grew to the maximum when adult and chick mortality were high. This is because when 'natural' host mortality was high

the parasite did not have time to build up to levels in individual grouse that that had a detrimental influence. Higher rates of larval mortality (0.4 larvae day^{-1}or more) prevented parasite burdens establishing in great numbers and the host always grew to the maximum. As before when parasite eggs developed more quickly the parasite was more likely to establish in the host and cause population extinction.

Analysis of the territorial model showed that the same pattern occurred (Figure 5.11a). Low larval mortality (less than 0.02 larvae day^{-1}) caused host population extinction. Increasing larval mortality to 0.1 larvae day^{-1} (figure 5.11b) allowed host population persistence except at the highest rates of host mortality when the host population persisted and the parasite population went extinct. Higher larval mortality (0.4 larvae day^{-1} or more) allowed host population persistence while the parasite population went extinct. When egg development to infective larvae was faster (7 days rather than 100 days) the host population always went extinct. Analysis of the territorial model showed that the same pattern occurred (Figure 5.10).

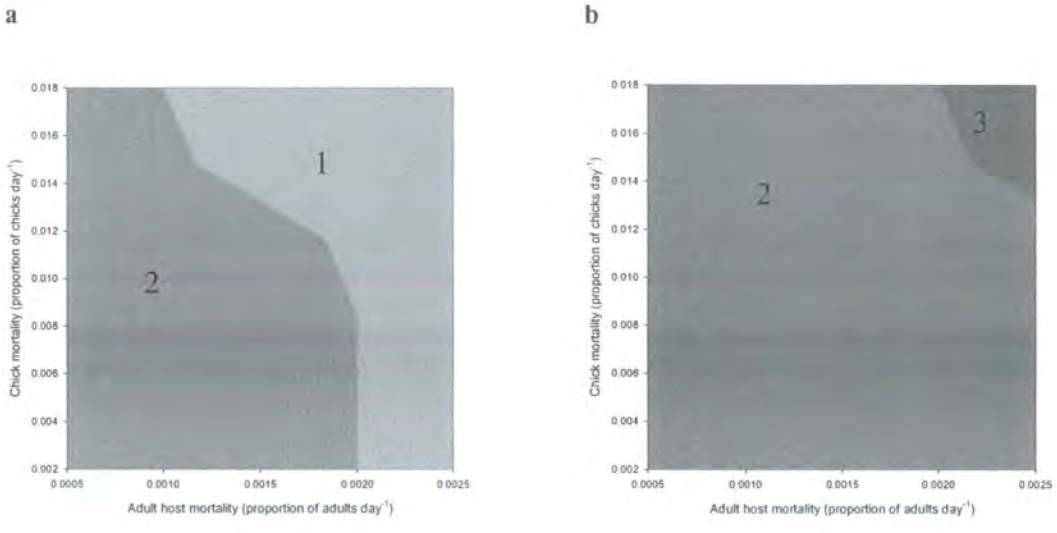


Figure 5.9 Effect of adult and chick mortality on the host population in the spatial model
Shaded areas indicate host population dynamics in the majority of replicates 1: host population extinction; 2: host population persistence for 50 years; 3: host population growth to maximum of 30000 individuals. Parameter values: egg mortality=0.1, larval ingestion=0.0001; rate of egg development=0.01; larval mortality= a 0.05, b 0.1

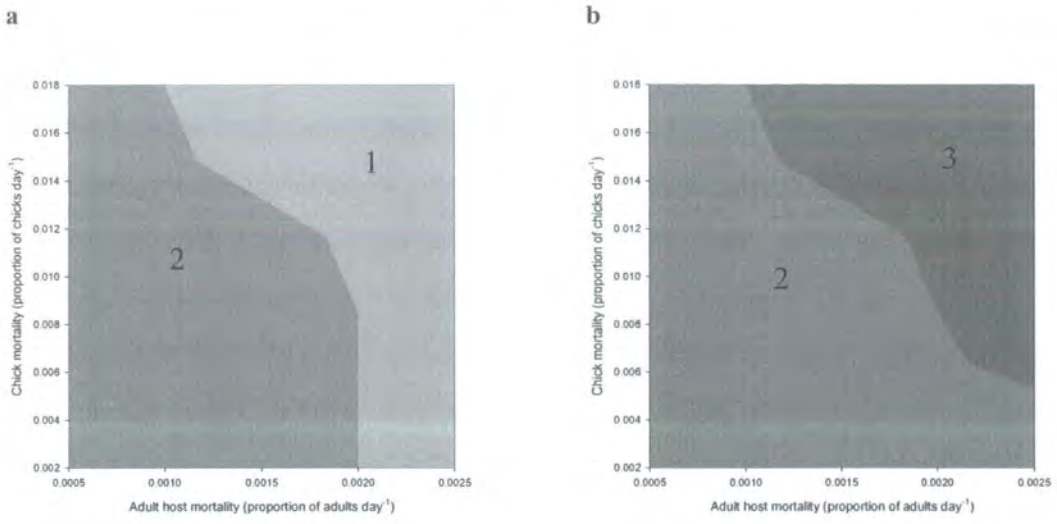


Figure 5.10 Effect of adult and chick mortality on the host population in the territorial model Shaded areas indicate host population dynamics in the majority of replicates 1: host population extinction; 2: host population persistence for 50 years; 3: host population persistence and parasite population extinction. Parameter values: egg mortality=0.1, larval ingestion=0.0001; rate of egg development=0.01; larval mortality = a 0.05, b 0.1.

SECTION 2 – ANALYSIS OF POPULATION CYCLICITY

5.4 METHODS

5.4.1 Host population persistence

Sensitivity analysis showed that some combinations of parameters allowed host population persistence for at least 50 years in the majority of 10 replicate tests. Persistence might indicate cycles in host population numbers and therefore the dynamics of these persistent populations were studied in more detail. A large number of parameter combinations caused host persistence (303 in the spatial model and 337 in the territorial model). In order to search for cyclicity I therefore focused on a small number of parameters concerning the parasite biology while keeping adult and chick mortality constant. Parasite parameters were studied in more detail because in control tests where the parasite was excluded the host population grew to the maximum number of individuals in the spatial model, indicating that the parasite had a regulatory effect on the host population. In addition previous modelling suggests that parasite larval biology (including larval mortality and time delays in development or transmission of infective stages of the parasite) influence the occurrence and period of host cycles (Anderson & May 1978; May & Anderson 1978; Dobson & Hudson 1992).

I reran the spatial and territorial models with a reduced parameter set, which included combinations I previously found to cause host persistence. I ran these combinations for a longer time period of 150 years, and again the host and parasite population sizes were output on a yearly basis. Two tests were conducted with two different sets of parameters (Table 5.4). The first test investigated the effect of egg mortality, larval mortality and the rate of egg development (468 combinations). The second tested the effect of larval mortality, larval ingestion and the rate of egg development (234 combinations). Each parameter combination was replicated 10 times. These tests produced time series that were then analysed for cyclicity (see section 5.4.2).

Spatial model control tests in Section 1 confirmed that the host always grew to the maximum when the parasite was excluded. However, in the territorial model in the absence of the parasite, the host population persisted due to density-dependent territorial

regulation. To be sure that the parasite was the cause of any host population cycles, those parameter combinations that did produce cyclic dynamics were tested in the model for 150 years without the parasite. The resulting time series were also analysed for cyclicity.

Table 5.4 Model parameters tested during sensitivity analysis

Parameter	Value, Test 1	Value, Test 2
Chick mortality (Proportion of chicks day^{-1})	0.0085	0.0085
Adult grouse mortality (Proportion of grouse day^{-1})	0.0015	0.0015
Parasite larval mortality (Proportion of larvae day^{-1})	0.0005, 0.005, 0.02, 0.05, 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9	0.0005, 0.005, 0.02, 0.05, 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9
Parasite egg mortality (Proportion of eggs day^{-1})	0.005, 0.02, 0.05, 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9	0.05
Larval ingestion (Proportion of larvae $grouse^{-1}$ day^{-1})	0.0001	0.00001, 0.00005, 0.0001, 0.0005, 0.001, 0.01
Parasite egg maturation to larvae (Proportion of eggs day^{-1})	0.01, 0.028, 0.14	0.01, 0.028, 0.14

5.4.2 Data analysis

Each replicate host population time series generated by the model (10 for each parameter combination) was analysed for cyclicity using time series autocorrelation following methods described by Diggle (1990). These methods have been used previously to analyse time series of red grouse populations (Potts *et al.* 1984; Williams 1985; Brown & Rothery 1994). This analysis requires roughly normal data without long term trends (Box *et al.* 1994). Any trend was removed by smoothing the time series (following Potts *et al.* (1984); Hudson (1992); Tapper (1992)). This was done by calculating a 5-year moving average which was itself averaged to produce an even smoother series. This procedure was repeated 10 times to produce a series that was subtracted from the original data (Figure 5.11). Using this method the 5-year average cannot be calculated for the first two and last two values each time the series is averaged. None of the time series showed any clear linear or non-linear trend over time, all trends were similar to that shown in figure 5.12. The detrended series were tested for normality using a Kolmogorov Smirnov test before analysis.

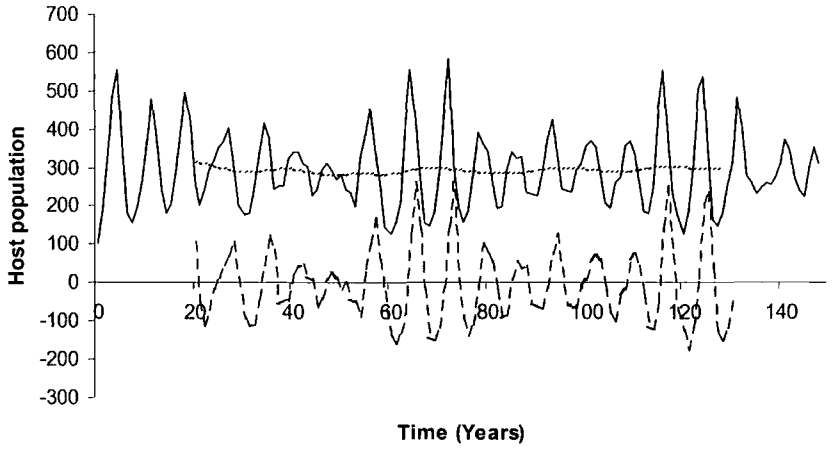


Figure 5.11 Smoothing of a host population time series by subtracting a smoothed time series. Original host population time series (—), 5 year moving average (- - -) and detrended host time series (– – –).

I tested for cyclicity using serial autocorrelation, which correlates the population at time t with the population at t+1L, t+2L....t+nL, where L is a time lag. The autocorrelation coefficient measures the correlation between successive observations. A series that shows cyclic fluctuations every six years will have significant positive correlations at the points in the cycle period of 6, 12, 18 years and negative correlations at 3, 9, 15 years.

A correlogram was produced by calculating autocorrelation coefficients for each detrended time series and plotted against time lag for lags of up to 60 years in SPSS (SPSS Inc. Chicago, USA). Figure 5.12 illustrates a) autocorrelation coefficients and b) partial autocorrelation coefficients of the smoothed host population time series shown in figure 5.11.

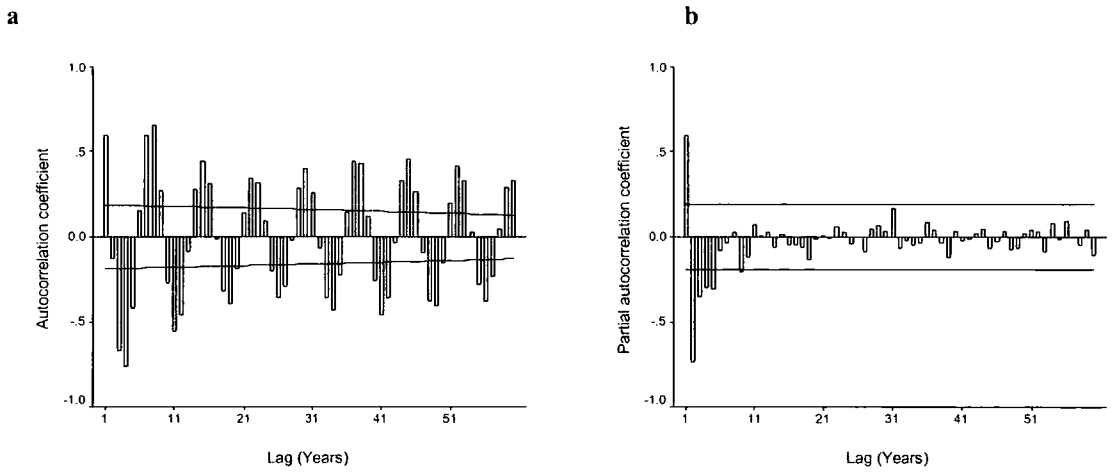


Figure 5.12 Correlogram illustrating a) autocorrelation coefficients b) partial autocorrelation coefficients for time lags up to 60 years. Bars represent the autocorrelation coefficients; horizontal lines show the 95% confidence limits.

The correlogram allows estimation of the statistical significance of the correlation coefficients by showing 95% confidence limits. Values greater than this can be regarded as significant at about the 5% level (Diggle 1990). For a completely random series the expected value of the correlation coefficient is 0 for time lags greater than zero. For a long random time series a coefficient of given lag will fall within the confidence intervals in about 95% of cases (Brown & Rothery 1993). For a cyclic time series with a sustained regular cycle the correlogram will show a corresponding regular cycle with similar frequency to the original time series (Brown & Rothery 1994). Real population fluctuations are never perfectly periodic and are therefore referred to as 'quasi cycles' (Nisbet & Gurney 1982). In a cyclic species, significant positive and negative correlation coefficients occur at each cycle and half-cycle for up to thirty years, with little or no damping (Moran 1952) and have been called 'phase remembering quasi cycles' (Nisbet & Gurney 1982).

Partial autocorrelation measures the correlations between two variables when the effect of a common correlation with a third variable has been excluded. i.e. partial autocorrelation between observations t and t2 is the autocorrelation between t and t2 after allowing for the autocorrelation between t and t2 and t1 and t (Brown & Rothery 1993). In figure 5.12b the partial autocorrelation coefficients show a relatively large negative value at lag 2 compared with the corresponding small negative autocorrelation.

So after allowing for the effect of a positive autocorrelation of lag year 1, the autocorrelation between observations two years apart is negative. After lags of 5 years the pattern of partial autocorrelation is irregular with small values. This indicates that the host numbers in one particular year are related to those in the previous 5 years.

Time series that are not strictly periodic, i.e. those that vary in the times between peaks and troughs have been termed 'phase forgetting quasi cycles'. Correlograms of these cycles have a damped appearance because with time variations in cycle length cause the correlation coefficients to weaken (Tapper 1992) (Figure 5.13). Cycles have been classed as heavily damped if they show a statistically significant negative correlation at time intervals corresponding to half a cycle without any subsequent significant positive correlation (Figure 5.14). These types of cycles have often been observed in the time series of wild red grouse (Potts *et al.* 1984; Williams 1985).

a Host population time series **b Correlogram of damped cycles**

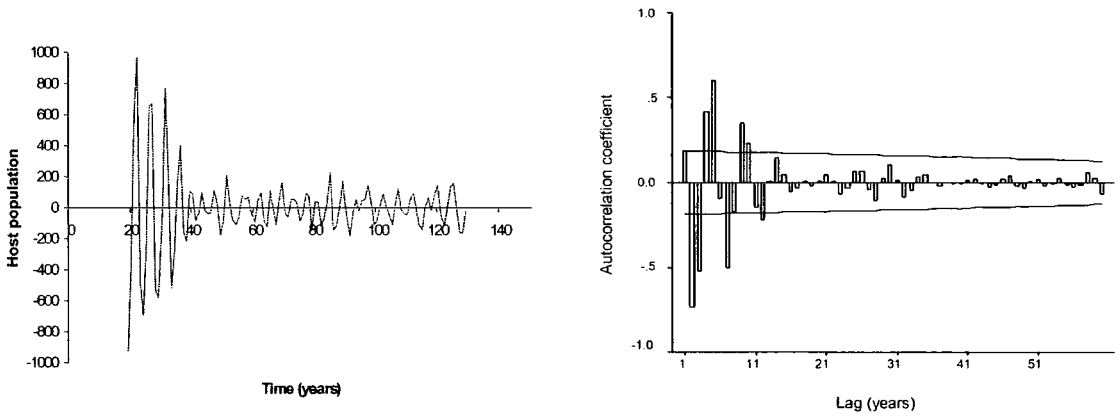


Figure 5.13 Time series and corresponding correlogram illustrating autocorrelation coefficients for time lags up to 60 years for a time series showing damped or diverging cycles. Bars in b represent the autocorrelation coefficients; horizontal lines show the 95% confidence limits.

a Host population time series **b Correlogram of heavily damped cycles**

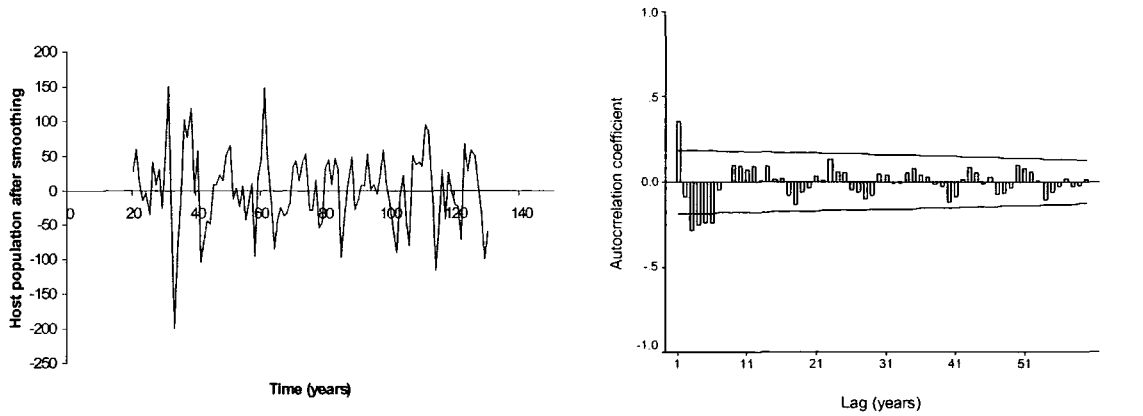


Figure 5.14 Model host population time series and corresponding correlogram illustrating autocorrelation coefficients for time lags up to 60 years for a time series showing heavily damped cycles. Bars in b represent the autocorrelation coefficients; horizontal lines show the 95% confidence limits.

The following criteria were used to assess the cyclicity of times series generated from the model tests. Time series were considered to be cyclic if there were significant positive and negative autocorrelation coefficients reflecting a complete cycle for at least 50 years. Time series were considered to be damped or 'phase forgetting' if there was at least one statistically significant positive and one significantly negative autocorrelation coefficient reflecting a full cycle, but the correlation coefficients damped to statistically insignificant values before 50 years. Time series were considered heavily damped if there was a significant negative correlation coefficient reflecting half a cycle period but no subsequent positive coefficient reflecting a full cycle period. The time series was not cyclic if none of the correlation coefficients were significant beyond a time lag of 1 year in 95% of cases. Each replicate time series was labelled as either cyclic, damped, heavily damped, or not cyclic as described above. Overall the dynamic for that parameter combination was the most common type of the 10 replicates.

The length of cycles was measured from the correlograms as the distance between the first and the next positive correlation coefficient. If there was no positive significant correlation coefficient then the cycle length was measured as double the lag to the first most significant negative correlation coefficient. The mean cycle period for one set of parameters was calculated from 10 replicates.

For parameter combinations where cycles occurred, a univariate GLM was used to test the influence of the variable parameters on the period of cycles. Residuals were tested for normality and cycle length was log transformed.

5.5 RESULTS

5.5.1 Host population stability

In the spatial model, 13.8% (97) parameter combinations allowed host persistence for 150 years. In those tests that did not complete 150 years, host populations grew to the maximum or went extinct within 50 years, although most tests ended in less than 20 years. This length of time was not long enough to determine whether host population showed cycles that eventually ended in host population extinction or extreme growth. In the territorial model 9.4% (66) tests completed 150 years. Those populations that went extinct always did so within 40 years (and most within 10 years). Some parameters caused parasite extinction while the host population reached a stable carrying capacity. In these cases the parasite population always went extinct within 50 years.

5.5.2 Cycle occurrence and cycle length

In both models all of the persistent populations exhibited cycles in host abundance. In the spatial model, 97% (94) of cycles were consistent regular cycles in the majority of replicates. Only 3% (3) of the cyclic time series within the parameter space tested showed damped cycles in the majority of the replicates, and none of the time series were heavily damped. In the territorial model 79% (52) of time series exhibited consistent cycles, 12% (8) were damped and the remaining 9% (6) were heavily damped. When the same parameter combinations were tested in the absence of the parasite none showed consistent cycles; however, some did produce damped cycles in the majority of replicates. In fact, 4% (3) showed damped cycles, 82% (54) showed heavily damped cycles and the remaining 14% (9) did not cycle. In general the cycles were weaker than those observed when the parasite was included. Time series that showed regular cycles when the parasite was present were damped (3), heavily damped (43) or not cyclic (6) with the parasite removed. Time series that were damped in the presence of the parasite were heavily damped (5) or not cylic (3) in the absence of the parasite. Time series that were heavily damped with the parasite remained heavily damped when it was removed from the model (6). The six times series that were heavily damped both with and without the parasite were removed from subsequent analysis, since the parasite may or may not contribute to the cyclic dynamics. The parasite increased the tendency of the

population to cycle in most tests, since although the populations showed damped cycles without the parasite, they exhibited more robust cycles when the parasite was present.

The cycle period ranged from 4.25 to 14.75 years in the spatial model and from 6.5 to 15.7 years in the territorial model. As an example, figure 5.15 illustrates the typical time series of host and adult parasites per host generated by the spatial model in 10 replicates of the same parameter tests.

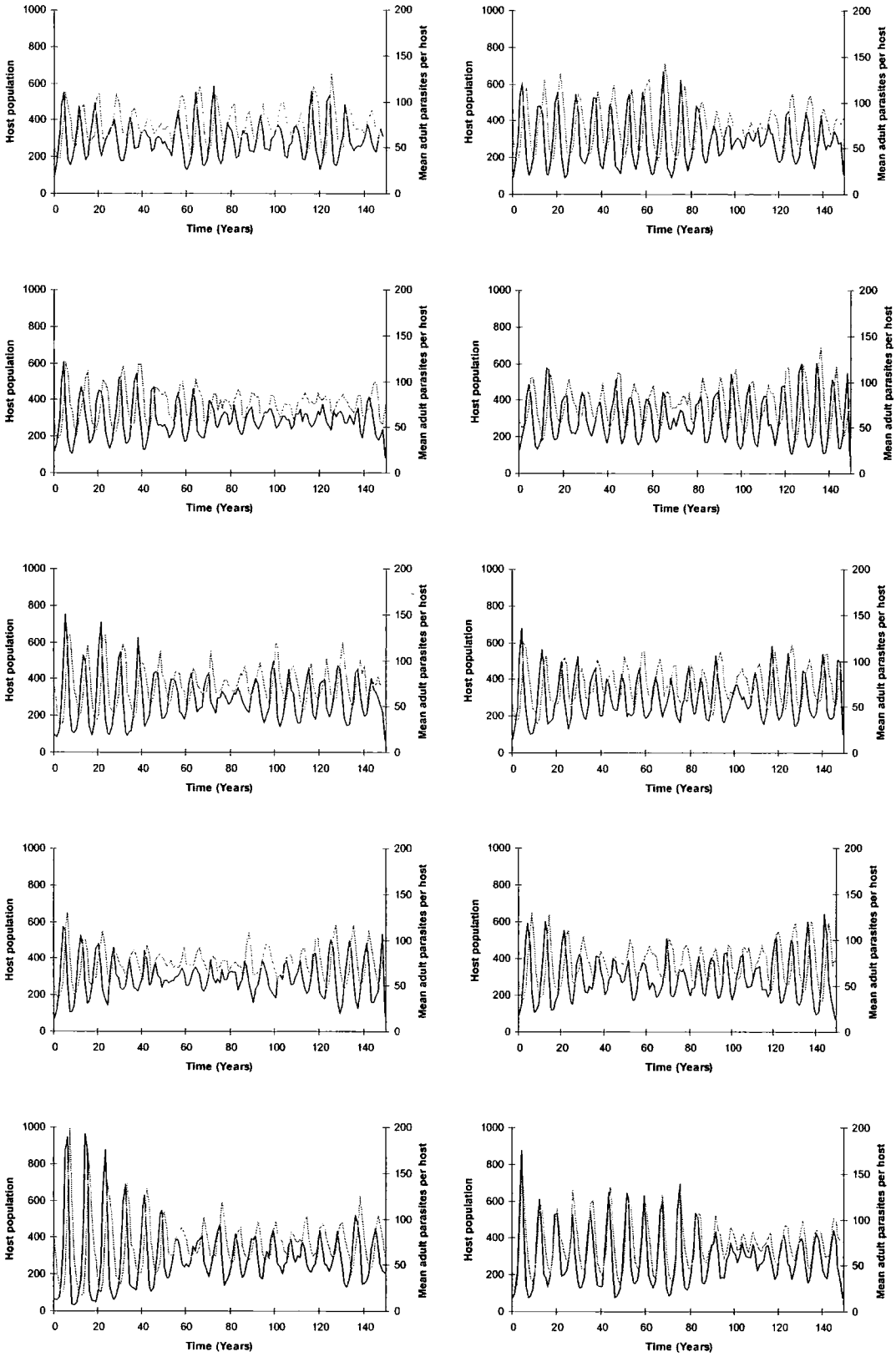


Figure 5.15 Host and parasite population numbers in 10 replicate model tests of one parameter combination in the spatial model. Host (black) and parasite (grey) population numbers were recorded yearly for 150 years. Parameter values: chick mortality=0.0085 chicks day^{-1}; adult host mortality=0.0015 adults day^{-1}; larval mortality=0.7 larvae day^{-1}; egg mortality=0.005 egg day^{-1}; larval ingestion=0.0001 larvae grouse^{-1} day^{-1}; rate of egg development=0.028 eggs day^{-1}

5.5.2.1 Influence of parasite biology parameters on the period of cycles

In the spatial model all parameters tested had a significant influence on the period of the model host population cycles. Period increased as larval mortality decreased ($F_{1,93}$=22.41, p<0.001), as egg mortality decreased ($F_{1,93}$=11.37 p=0.001), as rate of egg development increased ($F_{1,93}$=4.49, p=0.037) and as larval ingestion increased ($F_{1,93}$=17.47, p<0.001). In the territorial model none of the parameters had a significant influence on period of the host population cycle, (larval mortality: $F_{1,55}$=2.90, p=0.094; egg mortality: $F_{1,55}$=0.0.11 p=0.740); egg development: $F_{1,55}$=2.19, p=0.145; larval ingestion: $F_{1,55}$=2.65, p=0.109).

Parasite egg and larval mortality

Cycles occurred when parasite egg mortality or larval mortality was low (Figure 5.16a). In the spatial model period of cycles decreased a parasite larval mortality or parasite egg mortality increased. When parasite eggs developed quickly (Figure 5.16 a through to c), the parameter space where cycles occurred shifted to higher values of parasite mortality. Faster egg development compensated for higher parasite mortality. The length of the cycles did not appear to be affected. A similar pattern can be seen in the results from the territorial model (figure 5.17). The length of cycles was slightly different, in general cycles were longer than the equivalent parameter space in the spatial model.

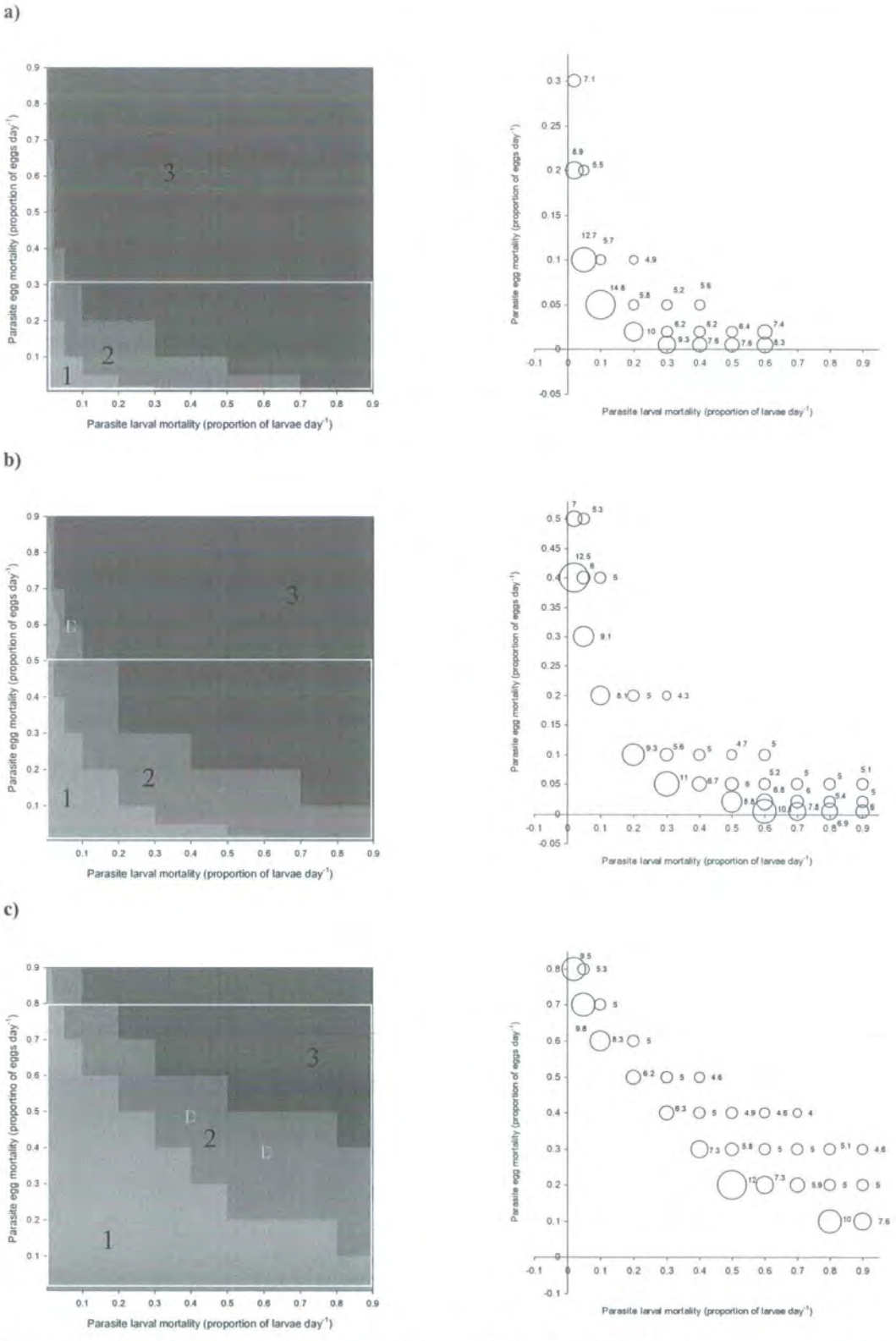


Figure 5.16 Spatial Model: Effect of parasite egg and larval mortality on the host population. *Left*: Contour plots illustrating population dynamics in the specified parameter space. Shaded areas indicate host population dynamics in the majority of replicates: 1: host population extinction; 2: host population persistence for 150 years; 3: host population growth to maximum of 30000 individuals. Parameter combinations that produced damped (D) or heavily damped (x) cycles have been pinpointed on the figures. These areas were not extrapolated because there were only a few cases in each graph where these types of cycles were recorded. *Right*: Cycle period. This illustrates the period of cycles in regions where they occur. The diameter of the bubble represents the cycle period. The white box on each contour plots illustrates the area shown in the corresponding bubble plot. Parameter values: larval ingestion=0.0001 larvae host^{-1} day^{-1}; rate of egg development = a 0.01 b 0.028 c 0.14 eggs day^{-1}

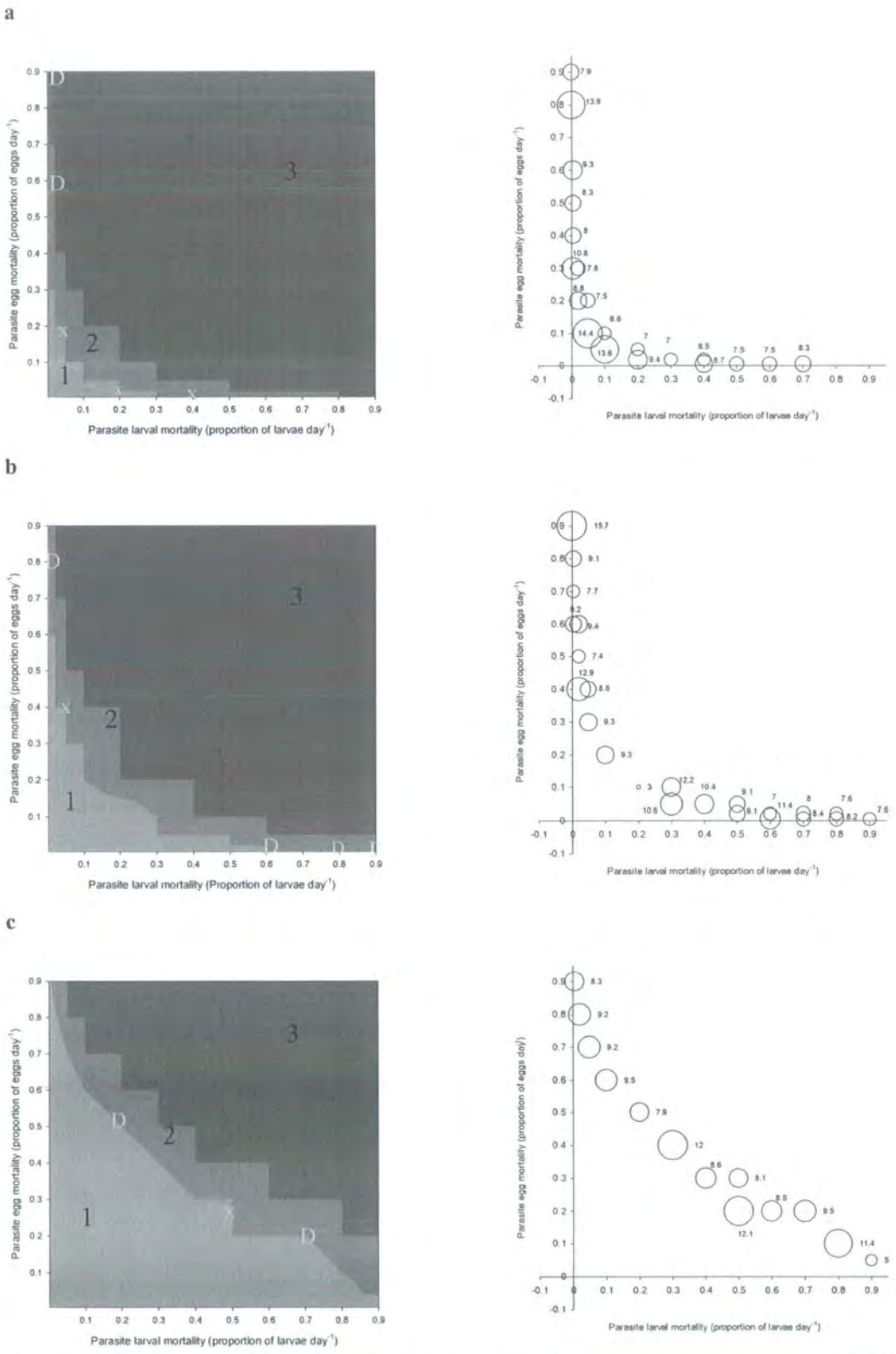
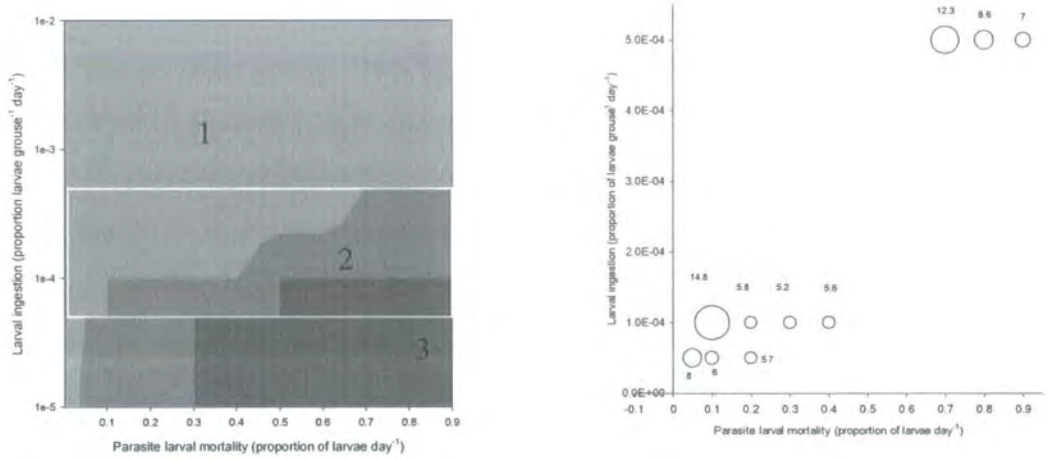


Figure 5.17 Territorial Model: Effect of parasite egg and larval mortality on the host population See Figure 5.16 for explanation of graphs. Parameter values: larval ingestion=0.0001 larvae host^{-1} day^{-1}; rate of egg development=a 0.01 b 0.028 c 0.14 eggs day^{-1}

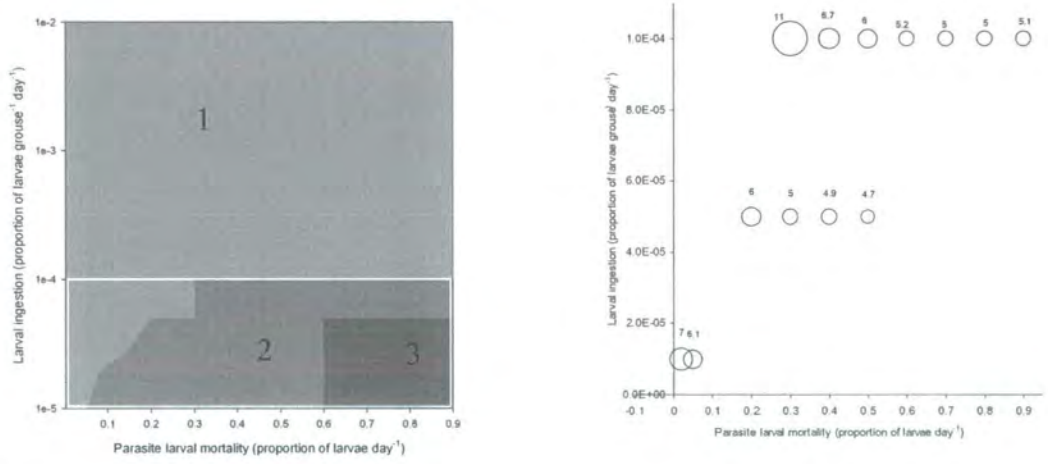
Parasite ingestion and larval mortality

Host population cycles occurred when larval ingestion was relatively low and larval mortality was high (Figure 5.18a). In the spatial model cycle period increased as larval ingestion increased and, as larval mortality decreased. In the spatial model length of cycles ranged from 5 to 12 years (figure 5.18a, b and c). When parasite eggs developed quickly (Figures 5.18a through to c), the parameter space where cycles occurred shifted to higher values of larval mortality and lower values of larval ingestion. Again faster egg development compensated for higher parasite mortality and lower larval ingestion. Cycle length was generally longer in the territorial model (Figure 5.19) compared to the spatial model for the same parameter space and ranged from 7 to 13 years (figure 5.19 a, b and c). As rate of egg development increased (figures a through c) the parameter space where cycles occurred shifted to higher values of larval mortality and lower values of larval ingestion.

a)



b)



c)

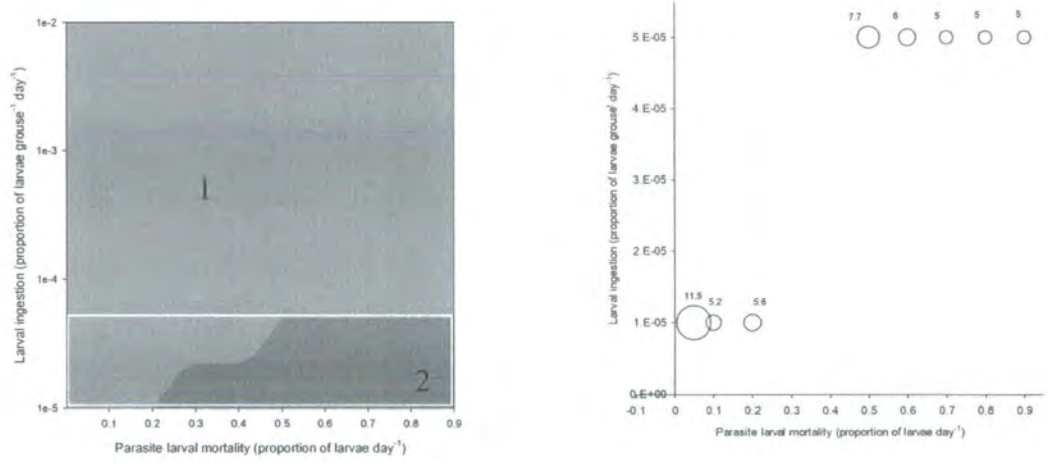
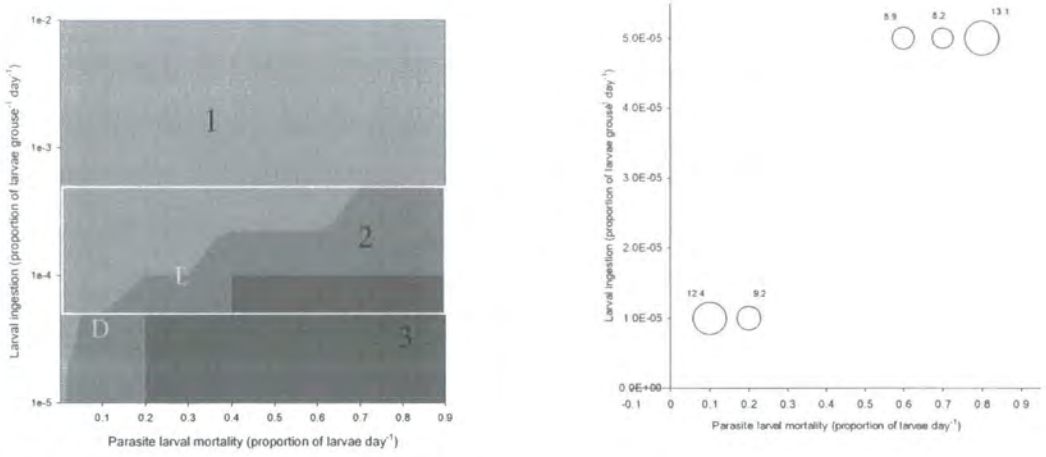
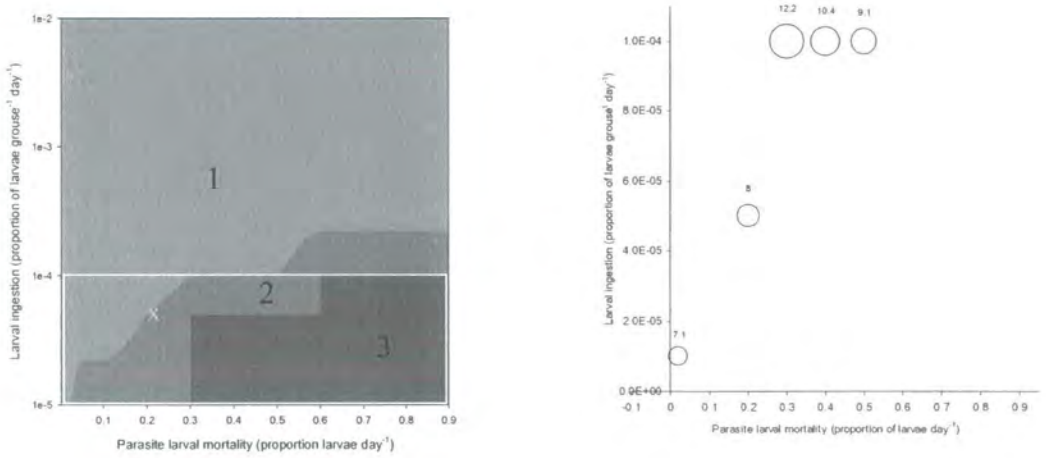


Figure 5.18 Spatial Model: Effect of larval ingestion and larval mortality on the host population See Figure 5.16 for explanation of graphs. Parameter values: parasite egg mortality=0.05 eggs day^{-1}; rate of egg development= a 0.01 b 0.028 c 0.14 eggs day^{-1}.

a)



b)



c)

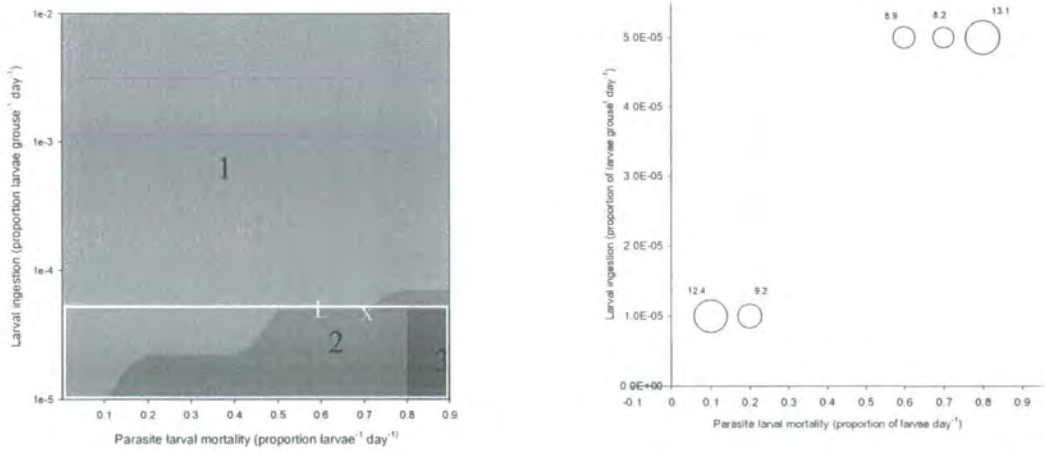


Figure 5.19 Territorial Model: Effect of larval ingestion and larval mortality on the host population dynamics. See Figure 5.16 for explanation of graphs. Parameter values: parasite egg mortality=0.05 eggs day^{-1}; rate of egg development= a 0.01 b 0.028 c 0.14 eggs day^{-1}

5.6 DISCUSSION

5.6.1 Red grouse population cycles

Individual-based stochastic spatial models using parameters for the red grouse-*T. tenuis* system generated host population time series showing cyclic changes in abundance. The parasite was the cause of these population cycles, since in the absence of the parasite the model host population either grew to the maximum allowed in the spatial model or reached the carrying capacity in the territorial model. While density-dependent mortality (through territorial behaviour) could generate damped cycles in some tests, the propensity of the population to cycle increased when the parasite was included. The results therefore support the hypothesis that the nematode parasite *T. tenuis* is an important factor influencing the cyclic patterns observed in grouse populations. However the absence of cyclicity in a simpler model lacking spatiality and territoriality suggests that these latter factors also play an important role.

The observed cycles ranged in length from 4 to 15 years, similar to those observed in wild grouse, where typical cycle periods of 3 to 5 years (Williams 1985; Potts *et al.* 1984; Hudson 1992; Hudson *et al.* 2002) and up to 15 years have been recorded (Hudson 1992; Haydon 2002). The amplitude of the cycles was also similar to those in red grouse population, with approximately 2 to 10 fold fluctuation in numbers (Moss & Watson 2001). One important point to note was that the parasite population peaked after the host population had begun to decline, a pattern which has been noted both in wild red grouse population cycles (Wilson 1983; Hudson *et al.* 1992b) and in previous modelling of the red grouse, *T. tenuis* interaction (Dobson & Hudson 1992). Thus the observed pattern of cyclicity suggests that establishing parasite burdens ultimately caused crashes in the host, and consequently the parasite population. However free-living larvae survived long enough to infect individuals when the host population started to recover after a crash.

Period of cycles changed according to the rates of egg and larval mortality, rate of parasite egg development and rate of larval ingestion. In the spatial model the cycle period decreased with increasing parasite egg and larval mortality; and increased with increasing rate of larval ingestion and rate of parasite egg development. This concurs

with previous models of host parasite interactions (May & Anderson 1978) and of the red grouse-*T. tenuis* interaction (Dobson & Hudson 1992). These similarly demonstrated that cycle period decreased as larval mortality increased. This result may be one reason that cycles do not occur in all wild red grouse populations, and, in addition, why cycle period (where cycles occur) varies between populations. *T. tenuis* egg and larval development, survival and transmission are dependent on environmental conditions such as humidity and temperature (Watson 1988; Shaw *et al.* 1989; Connan & Wise 1993, 1994). Dry conditions on some moors may cause high mortality of free-living parasite stages and prevent effective transmission to the host. Worm burdens would be unable to reach levels that have a regulatory influence on the host population. On other moors that are more humid free-living parasites can survive long enough for large worm burdens to build up. Indeed, cyclic populations do tend to be found in areas of high rainfall (Hudson 1992) (although some moors in drier areas do have cyclic host populations that may be caused by something other than the parasite (Hudson & Dobson 1990)). In addition, although not all grouse population cycles have been associated with high worm burdens (Watson *et al.* 1988) grouse from cyclic moors tend to have more parasites than those from non-cyclic moors. (On cyclic moors 24% of birds had more than 4000 worms compared to less than 4% on non cyclic moors (Hudson 1992)). When transmission is effective enough to cause cycles, length of cycles will be influenced by free-living parasite mortality, which in turn will be influenced by environmental conditions in that locality. In general, population cycles in northern Scotland are significantly longer than those in northern England (Hudson 1992; Hudson *et al.* 2002) although variation is thought to be due mostly to complex regional effects rather than latitude (Haydon *et al.* 2002).

The shape of the cycles produced by the models described here appeared to be similar to those on moors in Scotland, which are fairly symmetrical with increase, and decline phases approximately equal in length (Mackenzie 1952; Williams 1985; Moss *et al.* 1996). In northern England fluctuations are characteristically asymmetrical, with slow host population growth followed by a rapid decline (Potts *et al.* 1984; Hudson *et al.* 1992a; Hudson *et al.* 1998).

5.6.2 Model host population instability

Although a large proportion of the parameter combinations in the spatial model did result in population cycles, the majority caused host population instability. In real populations, resources such as food or space would limit both extinction and exponential growth, and the host population would be stabilised by density-dependent factors such as breeding or mortality. Including territoriality in the model introduced density-dependent host mortality. When transmission was too low for the parasite to be sustained in the host population, host numbers reached the carrying capacity. While territoriality tended to stabilise the host population, it generated damped cycles in some cases. However the tendency of population to cycle increased when the parasite was included. Interestingly, including density-dependent reduction in grouse fecundity and mortality in mathematical models of grouse populations also caused damped cycles in the absence of parasite-induced reductions in host fecundity (Dobson & Hudson 1992). Furthermore, reduction in parasite burdens in wild grouse populations can reduce the tendency for cyclic population crashes in host numbers demonstrating that the parasite plays a key role in the cycles (Hudson *et al.* 1998). However, there remained a residual cycle of greatly reduced amplitude, which could have been caused by some other mechanism. The territorial model suggests an explanation for the residual cycle recorded in those wild populations. In addition territorial behaviour is thought to be the cause of cycles in some wild red grouse populations (e.g. Matthiopoulos 1998, 2000; Mougeot *et al.* 2003) and this model suggests an explanation for cycles in other red grouse populations where parasite infections are too low to cause the observed cycles.

While territoriality did cause damped cycles in the host population in some cases, this intrinsic mechanism did not need to be present for grouse abundance to cycle. The spatial component to the population model was enough to generate host population cycles. The most likely explanation for this is that the spatial aspect allows some heterogeneity, for example a refuge for parasites. The spatial distribution of parasites was uneven (unlike in the population model) and therefore the aggregated parasite distribution among hosts stabilised the host population to a certain extent. This prevented extreme crashes or growth of the host population. Anderson & May (1978) demonstrated theoretically that aggregated parasite burdens have a stabilising effect on host populations. When this model was applied to the red grouse-*T. tenuis* system, the

relatively low levels of parasite aggregation observed in grouse populations (Hudson *et al.* 1992b; chapter 3) increased the tendency of the system to oscillate (Dobson & Hudson 1992). Host populations where parasites are highly aggregated are unlikely to cycle because the parasite-induced mortality would only happen to the few individuals with high infections (Anderson & May 1978). Inclusion of space in other population models has also been shown to influence their stability (Durrett & Levin 1994a, b; Hassell *et al.* 1994).

5.6.3 Comparison of model to conditions in wild red grouse populations

Both models produced cyclic dynamics in the host population for a wide range of realistic parameter values. Conditions causing cycles in the model are also likely to occur in wild red grouse populations. When conditions for parasite survival are good (in warm wet months), parasites eggs will develop quickly and egg and larval mortality will generally be low. When eggs took an average of 7 days to develop, cycles occurred for a wide range of egg mortality (0.1 to 0.9 eggs day^{-1}) and larval mortality (0.005 to 0.9 larvae day^{-1}). These values correspond to egg mortality recorded in laboratory experiments, which showed that minimum daily mortality of eggs, when development took approximately 7 days, was 0.09 eggs day^{-1}. In these tests eggs had adequate moisture and therefore, compared to field conditions, egg mortality is likely to be higher. In contrast, in winter conditions (from February to May) parasite eggs may take several weeks to develop (Connan & Wise 1993), and egg and larval mortality is likely to be high, although larvae are more resistant than eggs and significant numbers could survive winter temperatures (Connan & Wise 1993, 1994). Modelling showed that when egg development took approximately 100 days as it might in winter conditions, cycles occurred for a range of values of egg mortality (0.005 and 0.9 eggs day^{-1}) and larval mortality (0.0005-0.7 larvae day^{-1}). The highest rate of egg mortality at fluctuating temperatures, in laboratory experiments was 0.036 eggs day^{-1}, and larval mortality at fluctuating temperatures was much lower (0.002 larvae day^{-1}) (Connan & Wise 1993). Again moisture was sufficient and mortality is likely to be higher in the field. Nevertheless, cycles occurred for realistic parameter combinations where larval mortality was much lower than egg mortality.

Although the cycles produced in this model were of similar length to those in the wild, the mean host parasite burdens tended to be much lower than in wild grouse. In the model populations the mean parasite burdens were between 100 to 500 parasites. In real populations adult grouse tend to have infections in the region of thousands of parasites (see Chapter 3). Indeed a parasite burden of 30000 worms has been recorded (Wilson 1983; Hudson & Newborn 1987). This may simply indicate that the host mortality algorithm in the model was too severe. In fact May & Anderson (1978) suggested that mean parasite load may be a crude indication of the severity of the influence of a parasite (the smaller the mean, the more severe the parasite effect, assuming that the populations are in equilibrium). In the algorithm in this model the probability of parasite induced host mortality increased linearly with adult parasite burden up to a maximum of 30000 parasites (where probability of mortality = 1). In fact grouse body condition declines with a parasite burden of more than 3000 worms (Hudson 1986a) and although high burdens can result in death (Wilson & Wilson 1978; Hudson 1986a) the relationship between the number of adult worms in a bird and its condition is not clear (Jenkins *et al.* 1963). It may therefore be more appropriate to replace the linear correlation between host mortality and parasite burden with an algorithm describing a sigmoid relationship.

5.6.4 Conclusion

The individual-based spatial stochastic models presented here demonstrated that *T. tenuis* could generate cycles in grouse population numbers. The spatial aspect was of critical importance in allowing heterogeneity in the degree of host exposure to parasite infection. Density-dependent mortality stabilised the host population, and in some cases generated damped cycles in the host population in the absence of the parasite. Inclusion of the parasite increased the tendency for the host to cycle, reflecting the results of a long-term experimental manipulation of wild red grouse populations (Hudson *et al.* 1998). The realism of the model was supported by the observation that cycle periods were similar to those recorded in wild red grouse populations. Period varied with free-living parasite survival and development, which offers an explanation for differences in cycle length in red grouse populations in different regions of the UK.

CHAPTER 6

General discussion

6. GENERAL DISCUSSION

This thesis presents new data on the interaction between the red grouse and the nematode parasite *T. tenuis*. The detrimental effect of the parasite on individual birds and its role in regulating red grouse populations are of practical importance in red grouse management, in which the main aim is to maintain a high density of grouse. The work in this thesis is therefore pertinent to the efficacy of parasite control measures, the spatial interaction between parasite and host, and parasite induced host regulation. My findings also have wider implications in the fields of epidemiology and population ecology, adding support to the hypothesis that parasites can regulate host populations and may cause cycles in host abundance. This chapter discusses the findings in the context of the red grouse-*T. tenuis* interaction, and host-parasite interactions more generally, as well as suggesting directions for future research.

In a detailed individual-based model (Chapters 4 and 5) I demonstrated that *T. tenuis* could cause cycles in red grouse populations. Among many factors that can influence population cyclicity, these models particularly emphasised the importance of the spatial distribution of both the host and parasite, as cycles did not occur in non-spatial models. The primary effect of incorporating a spatial aspect was to increase the degree of aggregation of host infection, a finding which is key to understanding parasite-generated population cyclicity, since heterogeneous infection rates can destabilise host populations and lead to cycles (Anderson & May 1978; Dobson & Hudson 1992). Observations on wild grouse support this model, since free-living *T. tenuis* are aggregated both as eggs among caecal faeces (Chapter 3) and adults among hosts (Hudson *et al.* 1992b; Chapter 3) at a sufficiently heterogeneous level to generate host population cycles (Dobson & Hudson 1992). It is an important validation of the model that cycles were of similar wavelength to those recorded in wild grouse populations. Different parameter combinations led to variance in the cycle wavelength. This variance was associated with changes in parasite-related parameters, particularly transmission and survival. In the wild, these aspects of parasite biology are largely influenced by climate (Watson 1988; Shaw *et al.* 1989; Connan & Wise 1993, 1994). This raises the possibility that variations in the occurrence and wavelength of cycles in wild grouse populations across the UK could be explained by the differences in climate in different

geographic regions, which influence the survival and transmission of the parasite on different moors.

Models of biological systems are only as accurate as the data on which they are based. However I was able to construct models of the interaction between red grouse and *T. tenuis* that were relatively free of the problems of parameterisation, because such a large body of literature on the life history of both species now exists. By proceeding in a step-wise manner – beginning with a simple individual-based model before adding the spatial and territorial features – I was able to develop a model with a level of detail and realism that is often unachievable using analytical models (Bart 1995). Nevertheless, further refinements are always possible, and in at least one area the model requires further investigation: the parasite burdens that established in the model hosts were low compared to those in real cyclic red grouse populations. This is likely to indicate that the algorithm for linear parasite-induced host mortality was too severe. The algorithm could be modified to reflect the fact that grouse with less than about 3000 parasites do not appear to have a detrimental effect on host condition (Hudson 1986a). A linear or sigmoid relationship between host infection and host mortality, in which parasite burdens have little effect below 3000 parasites, may be more realistic.

The spatial distribution of parasites is of interest not only as a factor in the generation of population cycles, it also has considerable additional practical importance. In particular, the ability to accurately predict parasite infection would facilitate the targetting of management practices to reduce the number of parasites in specific areas of moorland and subpopulations of grouse. However, while the model highlighted the importance of the spatial and frequency distribution of parasites and hosts in host population dynamics, in fact little is currently known about the spatial distribution of free-living *T. tenuis* on moorland. Methods to quantify the infective larvae on vegetation are problematic (Saunders *et al.* 2000) and so I used an alternative approach of assessing parasite egg concentration in caecal faeces (Chapter 3). This demonstrated that while the infection intensity of individual grouse was related to the age and location of the host, egg concentration in caecal faeces was independent of all measured variables. Furthermore, there was only weak spatial autocorrelation in egg concentration of caecal

faeces, suggesting that the abundance of parasite eggs on the moor is largely unrelated to local environmental conditions – at least at the scale used here.

The local availability of eggs in caecal faeces is of interest because ultimately this limits the abundance of infective larvae, although I did not explicitly test this relationship. Successful transmission of infective larvae to the host will rely on a number of complex factors, which are likely to include the habitat or environmental conditions experienced by free-living parasites and the way in which these influence development and survival. Although I was unable to find any evidence of a spatial pattern in the distribution of parasite eggs, longer-term studies of this kind may reveal conditions that result in areas of high numbers of infective larvae, and determine whether certain areas are consistently highly infectious. While it is clear that moisture and temperature influence yields of *T. tenuis* larvae, the conditions influencing migration of larvae and host infection are less well known. For example, greatest yields of larvae occur at 20°C in laboratory tests, yet at this temperature, there is less larval migration than at cooler temperatures (Saunders *et al.* 2000).

Both theoretical (Dobson & Hudson 1992; Chapter 5) and experimental (e.g. Hudson *et al.* 1998) approaches have shown that *T. tenuis* has a significant impact on the population dynamics of red grouse. This has become one of the best-studied examples of host population regulation by parasites, and there is comparatively little empirical evidence regarding the role of parasites in other cycling species. This may be because sub-lethal effects of parasites on host fecundity may have caused their impact to be overlooked (Dobson & Hudson 1992). The small number of studies which do exist have focussed on the role of macroparasites (nematode species), for example in snowshoe hares (Ives *et al.* 1997) and reindeer (Albon *et al.* 2002). Cycles do occur in other grouse species (for review see Lindstrom 1995) but parasites are unlikely to be a factor here, since *T. tenuis* burdens are typically low in populations outside of Britain (Moss & Watson 2001). In comparison, there are few studies on the role of microparasites (for review see Hudson *et al.* 2002). Recently, research on field vole populations (*Microtus agrestis*) indicated that prevalence of the cowpox virus and clinical signs of tuberculosis (*Mycobacterium microti*) rose as vole numbers increased, and peaked as numbers declined (Cavanagh *et al.* 2004). There was a lag in infection prevalence in response to

changes in host abundance, suggesting that the disease plays an underestimated role in the dynamics of cyclic populations.

Although *T. tenuis* plays a crucial role in the population cycles of red grouse, the results of individual-based models also emphasise the need to consider other mechanisms unrelated to parasitism (Chapters 4 and 5). I found density-dependent host mortality was sufficient to stabilise the host population, and that some parameter combinations generated host population cycles even when the parasite was removed from the system. Including the parasite increased the magnitude of the cycles, which supports a role for parasitism in host cyclicity, but this example illustrates the underlying complexity of host population cycles. Similar results have been obtained in wild red grouse populations, in which experimental parasite removal reduced the extent of population crashes. While the parasite was the cause of the cycles, there was still a tendency to cycle in numbers with the parasite removed (Hudson *et al.* 1998). Clearly parasitism is unlikely to be the explanation for red grouse cycles in every population. Furthermore, where the parasite is involved in regulating host population dynamics, it is possible that it does so in association with a number of other factors.

A particularly important variable influencing population dynamics is the spacing behaviour of grouse, a mechanism that has been hypothesised to cause cycles in some red grouse populations (e.g. Mountford *et al.* 1990; Moss & Watson 1991; Moss *et al.* 1996; Mougeot *et al.* 2003). The factors underlying population cycles need not be mutually exclusive, however there has been little overlap in the study of the roles of the parasite and of spacing behaviour. To date studies into the spacing behaviour hypothesis have rarely addressed the influence of *T. tenuis* (Moss *et al.* 1993b) and have generally concentrated on male grouse. In comparison, research on the parasite has been concerned with the influence on female grouse, while the impact of *T. tenuis* on male behaviour and body condition has been largely ignored (Fox 1999, Fox & Hudson 2001). It remains a possibility that both the territoriality and parasite-regulation hypotheses for grouse cyclicity are correct, but that each applies in different populations (Moss *et al.* 1993). Research on spacing behaviour has mostly been conducted on typically drier Scottish grouse moors with low parasite burdens (Moss & Watson 2001), while research on the parasite has mostly been conducted on the wetter moors in

northern England where parasite burdens tend to be higher (Hudson 1992). However, regional specificity is not supported by recent work demonstrating that intrinsic processes (experimentally increased aggressiveness) can influence red grouse population dynamics across both England and Scotland (Mougeot *et al.* 2003). The experiment showed that increased aggression in autumn reduced recruitment and subsequent breeding density, and that population trajectories switched from increasing to declining.

Alternatively, both mechanisms could be operating simultaneously (e.g. Krebs 1995; Ives & Murray 1997; Gilg *et al.* 2003) or the cycles could be caused by one dominant mechanism, which varies geographically (Berryman 2002). Indeed parasites have been shown to interact with testosterone to reduce the level of aggressive behaviour in red grouse (Fox & Hudson 2001). These authors rejected the theory that the two mechanisms are synergistic however, on the basis that at peak host population density the hypotheses predict conflicting directions in male aggressiveness: increasing aggressiveness according to the spacing hypothesis, and declining aggressiveness with parasite hypothesis (Fox & Hudson, 2001). Future research should be directed at testing the conditions under which each mechanism dominates the system, and how they may interact to generate unstable population dynamics (Mougeot *et al.* 2003). Modelling which incorporates both parasites and spacing behaviour simultaneously would help in understanding the complexities of the interactions. This is exactly the sort of model I presented in Chapter 5, although this could be further refined to include territory size, relatedness between individuals, and differential aggression between kin and non-kin. These aspects of grouse behaviour have been considered in discrete models based on age structured populations (Matthiopoulos *et al.* 1998) or kin clusters (Matthiopoulos *et al.* 2000) and individual-based spatially explicit models (Hendry *et al.* 1997); however none of these models incorporated the influence of *T. tenuis.*

It is not always necessary or advisable to model all aspects of a biological system, however a number of factors that could influence host population dynamics were not considered in the present model. One such aspect is parasite mortality. The models described in Chapters 4 and 5 assume a constant rate of parasite egg and larval mortality throughout the year, although I tested a wide range of values for these parameters. In

reality, however, survival and development of *T. tenuis* varies seasonally (Shaw 1988; Shaw *et al.* 1989; Connan & Wise 1993, 1994), and in addition larvae can arrest development (Gibbs 1986; Shaw 1988; Shaw *et al.* 1989). Short periods of larval arrestment (2 months) have previously been shown to increase the tendency for population cycles to develop, while longer periods of arrestment (more than 6 months) can lead to damped cycles or the complete absence of cycles (Dobson & Hudson 1992). In this model, proportional arrestment and seasonal survival of free-living parasites both operate to perturb the system sufficiently to maintain cycles (Dobson & Hudson 1994)

Other factors that can influence host population dynamics, but which have yet to be explicitly modelled, include the impact of shooting, predators and parasite control regimes. In these cases, modelling may be useful to generate hypotheses regarding grouse management for subsequent testing in field experiments. Mortality of birds through shooting may be compensated for by increased survival of the remaining birds (Hudson & Watson 1985), however it does not appear to stabilise the population dynamics of the harvested grouse population (Hudson *et al.* 2002). Modelling could thus be used to improve understanding of alternative shooting strategies (e.g. Willebrand & Hornell 2001). Predation should also be considered, because *T. tenuis* can increase predation risk of red grouse, which in turn reduces the tendency for oscillations (Hudson *et al.* 1992a; Thirgood *et al.* 2000; Hudson *et al.* 2002). This is thought to result from the selective removal of a few heavily infected hosts reducing the delayed density-dependent effects of parasites on host survival and breeding. Conversely there is also evidence that predators can generate cycles in their prey: for instance, the specialist gyrfalcon (*Falco rusticolus*) can drive cycles in abundance of ptarmigan (*Lagopus mutus*) in Iceland (Nielsen 1999). Furthermore with respect to parasite control, modelling predictions suggest that treatment of hosts to control a parasite may alter host population dynamics, with treatment preventing parasite-regulation of the host population. Such a change can result in an increase in both host and parasite populations, and may not even reduce the mean parasite burden per host (May & Anderson 1978). While current methods of parasite control are effective at reducing parasite burdens (Chapter 2), it is possible that resistance to anthelmintics could develop in the future. Modelling could help to assess the consequences (e.g. Barnes *et al.* 1995).

A final refinement to the model would be the introduction of landscape heterogeneity, which may go some way to enhancing the realism of the model, although this explicitness can make models very complex (Ruckelshaus *et al.* 1997; Ginzberg & Jensen 2004). Clearly the spatial distribution of host and parasite is important in the occurrence of population cycles (Chapter 5) and assessing the dynamics of red grouse and *T. tenuis* in a simple, yet heterogeneous habitat, may provide further insight into the causes of the cycles. Information detailing the spatial distribution of free-living parasites (Chapter 3), or red grouse (e.g. Palmer & Bacon 2001) on an area of moorland could then be included. Spatially explicit population models have successfully simulated for example, the dynamics of the parapoxvirus disease in grey squirrels (Rushton *et al.* 2000). Clearly, careful evaluation of individual-based models is necessary before they can be used to make management decisions (e.g. Bart 1995).

By using a combination of novel modelling approaches and field data, I was able to help clarify the role of the parasite *T. tenuis* in the regulation of red grouse populations. This not only benefits management practices specific to this important game species, it also advances our understanding of host-parasite interactions and parasite-induced population cycles. Red grouse and *T. tenuis* provide an excellent study system, and will undoubtedly continue to be a key research focus in the analysis of population dynamics of parasite populations and their hosts.

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Appendices

APPENDIX 1

Visual Basic program code for the individual-based population model described in chapter 4.

Notes explaining sections of code are preceded by an apostrophe

```
Option Explicit
'INDIVIDUAL BASED STOCHASTIC MODEL OF RED GROUSE AND T. TENUIS

'Initial conditions and fixed parameters
Const startg As Integer = 100                    'starting number of grouse
Const startp As Integer = 100                    'starting number of parasites in grouse
Const startW As Integer = 0                      'starting number of free living parasites
Const starte As Integer = 0                      'starting number of free parasite eggs
Const maxyear As Integer = 50                    'maximum number of years
Const repday As Integer = 238                    'day grouse reproduce
Const independenceday As Integer = 305           'day when chicks become adults
Const fecund As Integer = 8                      'no. chicks per host
Const pfecund As Integer = 130                   'parasite fecundity
Const probdinhost As Single = 0.003              'adult parasite mortality
Const maxg As Integer = 30000                    'maximum number of grouse
Const mingrouse As Integer = 0                   'minimum number of grouse
Const maxchicks = 50000                          'maximum number of chicks
Const maxpinhost = 30000                         'maximum parasites in one grouse

'Variable parameters
Dim probcd As Single          'proportion of chicks dying- not parasite-induced
Dim probadnatural As Single 'proportion of adult grouse dying, not parasite-induced
Dim probdiew As Single        'proportion of free living parasite larvae dying
Dim probdiee As Single        'proportion of parasite eggs dying
Dim pickup As Single          'proportion of free living parasite host picks up
Dim rate As Single            'proportion of parasite eggs developing to larvae

'Other variables
'c=counter
Dim rep As Integer                               'no of reps of the program
Dim iprobcd As Integer                           'c
Dim iprobadnatural As Integer                    'c
Dim iprobdiew As Integer                         'c
Dim iprobdiee As Integer                         'c
Dim ipickup As Integer                           'c
Dim irate As Integer                             'c
Dim nchick(1 To maxg) As Integer                 'no chicks per adult grouse
```

```
Dim alive(1 To maxg) As Integer                'c
Dim pingrouse(1 To maxg) As Single             'no. parasites per adult grouse
Dim pinchick(1 To maxg) As Single              'no. parasites per chick
Dim currentg As Integer                        'c
Dim year As Integer                            'c
Dim day As Integer                             'c
Dim currentw As Single                         'c
Dim currente As Single                         'c
Dim totaladults(1 To 365) As Single            'no. adults per day
Dim totalchicks(1 To 365) As Single            'no. chicks per day
Dim totalparasites(1 To 365) As Single         'no. adult parasite per day
Dim freeliving(1 To 365) As Single             'no. larvae per day
Dim eggs(1 To 365) As Single                   'no. eggs per day
Dim finish As Integer                          'c
Dim violation As Integer                       'c
Dim ott As Integer                             'c
```

Sub calculate() 'calculation of population numbers

```
Dim g As Integer

totaladults(day) = currentg
freeliving(day) = currentw
eggs(day) = currente
totalchicks(day) = 0
totalparasites(day) = 0

For g = 1 To currentg
If nchick(g) = 0 Then totalparasites(day) = totalparasites(day) + pingrouse(g)
If nchick(g) > 0 Then
totalparasites(day) = totalparasites(day) + pingrouse(g) + (nchick(g) * pingrouse(g))
'assuming all have same no of parasites
totalchicks(day) = totalchicks(day) + nchick(g)
If (totalchicks(day) > maxchicks) Then totalchicks(day) = maxchicks And ott = 1
End If
Next g
```

End Sub 'calculate

Sub initialconditions() 'initial model conditions

```
Dim g As Integer

currentg = startg
currentw = startW
currente = starte
violation = 0
ott = 0
```

```
For g = 1 To currentg
pingrouse(g) = startp
nchick(g) = 0
pinchick(g) = 0
Next g

End Sub 'initialconditions

Sub deathinhost() 'adult parasite mortality
Dim g As Integer

For g = 1 To currentg
pingrouse(g) = pingrouse(g) - (probdinhost * pingrouse(g))
If nchick(g) > 0 Then pinchick(g) = (pinchick(g) - (probdinhost * pinchick(g)))
Next g

End Sub 'deathinhost

Sub spreadparasite() 'parasite reproduction
Dim g As Integer

For g = 1 To currentg
If pingrouse(g) >= 0 And nchick(g) = 0 Then
eggs(day) = eggs(day) + (pingrouse(g) * pfecund)
End If

If pingrouse(g) >= 0 And nchick(g) > 0 Then
eggs(day) = eggs(day) + (pingrouse(g) * pfecund) + (nchick(g) * pinchick(g) * pfecund)
End If
Next g

End Sub 'spreadparasite

Sub pickupparasite() 'parasite ingestion
Dim g As Integer
Dim prop As Single

If (freeliving(day) - ((currentg + totalchicks(day)) * pickup * freeliving(day))) >= 0
Then prop = pickup
If (freeliving(day) - ((currentg + totalchicks(day)) * pickup * freeliving(day))) < 0 Then
prop = (1 / (currentg + totalchicks(day)))

'If not enough larvae for all grouse, then larvae are divided between all hosts
'adult and chick ingest at the same rate at the moment

For g = 1 To currentg
pingrouse(g) = pingrouse(g) + prop * freeliving(day)
If pingrouse(g) > maxpinhost Then pingrouse(g) = maxpinhost
If nchick(g) > 0 Then pinchick(g) = pinchick(g) + prop * freeliving(day)
If pinchick(g) > maxpinhost Then pinchick(g) = maxpinhost
```

```
Next g
freeliving(day) = freeliving(day) - ((currentg + totalchicks(day)) * (prop *
freeliving(day)))

End Sub 'pickupparasite

Sub freelivingdie() 'parasite larval mortality
freeliving(day) = freeliving(day) - (probdiew * freeliving(day))

End Sub 'freelivingdie

Sub eggsdie() 'parasite egg mortality
eggs(day) = eggs(day) - (probdiee * eggs(day))

End Sub 'eggsdie

Sub diegrouse() 'grouse mortality
Dim ndead, g, r As Integer

ndead = 0
For g = 1 To currentg
alive(g) = 1
Next g

For g = 1 To currentg
If (probadnatural > Rnd) Or ((pingrouse(g) / maxpinhost) > Rnd) Then
alive(g) = 0
ndead = ndead + 1
End If
If (alive(g) = 0) Then pingrouse(g) = 0
If (day > repday) And (day < independenceday) And (alive(g) = 0) Then
nchick(g) = 0
pinchick(g) = 0
End If
Next g

'update grouse, parasites and chicks

r = 0
currentg = currentg - ndead

For g = 1 To currentg
Do
r = r + 1
Loop Until (alive(r) = 1)
pingrouse(g) = pingrouse(r)
If (day > repday) And (day < independenceday) Then
nchick(g) = nchick(r)
pinchick(g) = pinchick(r)
End If
```

```
Next g

End Sub 'diegrouse

Sub reproduce() 'grouse reproduction
Dim g As Integer

For g = 1 To currentg
nchick(g) = fecund 'all have same no. of chicks
pinchick(g) = 0
Next g

End Sub 'reproduce

Sub diechick() 'chick mortality
Dim g, i, nchk As Integer

For g = 1 To currentg
If nchick(g) > 0 Then
nchk = nchick(g) 'seperating chicks into individuals
For i = 1 To nchk
If probcd > Rnd Or ((pinchick(g) / maxpinhost) > Rnd) Then nchick(g) = nchick(g) - 1
Next i
End If
Next g

End Sub 'diechick

Sub independence() 'chicks mature to adult grouse
Dim c, g, nr, i As Integer

violation = 0
c = 0
For g = 1 To currentg
If nchick(g) > 0 Then
c = c + nchick(g) 'counting total no. of mature chicks
End If
Next g

nr = 0
For g = 1 To currentg
If nchick(g) > 0 Then
For i = 1 To nchick(g)
nr = nr + 1
If (currentg + nr) <= maxg Then pingrouse(currentg + nr) = pinchick(g)
If (currentg + nr) > maxg Then violation = 1

Next i
End If
Next g
```

```
If violation = 0 Then
currentg = currentg + c 'adding chicks to adult population

For g = 1 To currentg
nchick(g) = 0
pinchick(g) = 0
Next g
End If

End Sub 'independence

Sub mature() 'parasite egg development to larvae
Dim ne As Single

ne = eggs(day) - (rate * eggs(day))
freeliving(day) = freeliving(day) + rate * eggs(day)
eggs(day) = ne

End Sub 'mature

Sub wfile() 'data output file
Dim g, totalday As Integer

If rep = 1 Then
If ipickup = 1 And iprobdiee = 1 And iprobdiew = 1 And iprobadnatural = 1 And
iprobcd = 1 And irate = 1 Then Open "c:\Ruth\Basic1.txt" For Output As #1
If ipickup > 1 Or iprobdiee > 1 Or iprobdiew > 1 Or iprobadnatural > 1 Or iprobcd > 1
Or irate > 1 Then Open "c:\Ruth\Basic1.txt" For Append As #1
End If

If rep = 2 Then
If ipickup = 1 And iprobdiee = 1 And iprobdiew = 1 And iprobadnatural = 1 And
iprobcd = 1 And irate = 1 Then Open "c:\Ruth\basic2.txt" For Output As #1
If ipickup > 1 Or iprobdiee > 1 Or iprobdiew > 1 Or iprobadnatural > 1 Or iprobcd > 1
Or irate > 1 Then Open "c:\Ruth\basic2.txt" For Append As #1
End If

If rep = 3 Then
If ipickup = 1 And iprobdiee = 1 And iprobdiew = 1 And iprobadnatural = 1 And
iprobcd = 1 And irate = 1 Then Open "c:\Ruth\basic3.txt" For Output As #1
If ipickup > 1 Or iprobdiee > 1 Or iprobdiew > 1 Or iprobadnatural > 1 Or iprobcd > 1
Or irate > 1 Then Open "c:\Ruth\basic3.txt" For Append As #1
End If

If rep = 4 Then
If ipickup = 1 And iprobdiee = 1 And iprobdiew = 1 And iprobadnatural = 1 And
iprobcd = 1 And irate = 1 Then Open "c:\Ruth\basic4.txt" For Output As #1
If ipickup > 1 Or iprobdiee > 1 Or iprobdiew > 1 Or iprobadnatural > 1 Or iprobcd > 1
Or irate > 1 Then Open "c:\Ruth\basic4.txt" For Append As #1
```

```
End If

If rep = 5 Then
If ipickup = 1 And iprobdiee = 1 And iprobdiew = 1 And iprobadnatural = 1 And
iprobcd = 1 And irate = 1 Then Open "c:\Ruth\basic5.txt" For Output As #1
If ipickup > 1 Or iprobdiee > 1 Or iprobdiew > 1 Or iprobadnatural > 1 Or iprobcd > 1
Or irate > 1 Then Open "c:\Ruth\basic5.txt" For Append As #1
End If

If rep = 1 And ipickup = 1 And iprobdiee = 1 And iprobdiew = 1 And iprobadnatural =
1 And iprobcd = 1 And irate = 1 Then
Print #1, "maxyear", maxyear
Print #1, "maxg", maxg
Print #1, "startg", startg
Print #1, "startp", startp
Print #1, "startw", startW
Print #1, "starte", starte
Print #1, "maxpinhost", maxpinhost
Print #1, "repday", repday
Print #1, "independenceday", independenceday
Print #1, "fecund", fecund
Print #1, "pfecund", pfecund
Print #1, "probdinhost", probdinhost
Print #1, " ", "finish", " ", "ott", "  ", "year", "  ", "day", "  ", "totaladults", "  ",
"totalchicks", "  ", "totalparasites", "  ", "freeliving", "  ", "eggs", "  ", "rate", " ",
"probcd", " ", " probadnatural", " ", "probdiew", " ", "probdiee", " ", "pickup"
End If

Print #1, finish, " ", ott, " ", year, "  ", day, "  ", totaladults(day), "  ", totalchicks(day),
" ", totalparasites(day), " ", freeliving(day), "  ", eggs(day), "  ", rate, "  ", probcd, "  ",
probadnatural, "  ", probdiew, "  ", probdiee, "  ", pickup
Close #1

End Sub 'wfile

Private Sub cmdQuit_Click() 'quit button
End
End Sub 'cmdQuit_Click

'START OF PROGRAM

Private Sub cmdStart_Click() 'start button

For rep = 1 To 10

Randomize

For irate = 1 To 4
If irate = 1 Then rate = 0.01
If irate = 2 Then rate = 0.02
```

```
If irate = 3 Then rate = 0.04
If irate = 4 Then rate = 0.14
'if rate = 0.14 parasite eggs take an average of 7 days to develop
'if rate = 0.01 parasite eggs take an average of 100 days to develop

For iprobcd = 1 To 3
If iprobcd = 1 Then probcd = 0.002
If iprobcd = 2 Then probcd = 0.0085
If iprobcd = 3 Then probcd = 0.018
'if probcd=0.002 then 87% chicks survive to adulthood
'if probcd=0.018 then 30% survive to adulthood

For iprobadnatural = 1 To 3
If iprobadnatural = 1 Then probadnatural = 0.0005
If iprobadnatural = 2 Then probadnatural = 0.0015
If iprobadnatural = 3 Then probadnatural = 0.0025
'if probadnatural = 0.0005 then 83% of adult grouse survive one year
'if probadnatural = 0.0025 then 40% survive one year

For iprobdiew = 1 To 7
If iprobdiew = 1 Then probdiew = 0.0005
If iprobdiew = 2 Then probdiew = 0.005
If iprobdiew = 3 Then probdiew = 0.02
If iprobdiew = 4 Then probdiew = 0.05
If iprobdiew = 5 Then probdiew = 0.1
If iprobdiew = 6 Then probdiew = 0.4
If iprobdiew = 7 Then probdiew = 0.8
'If probdiew=0.0005 then 17% of larvae die in one year
'If probdiew=0.8 then 99% of larvae die within 3 days

For iprobdiee = 1 To 6
If iprobdiee = 1 Then probdiee = 0.005
If iprobdiee = 2 Then probdiee = 0.02
If iprobdiee = 3 Then probdiee = 0.05
If iprobdiee = 4 Then probdiee = 0.1
If iprobdiee = 5 Then probdiee = 0.4
If iprobdiee = 6 Then probdiee = 0.8
'If probdiee=0.005 then 845 of eggs die in one year
'If probdiee=0.8 then 99% of eggs die within 3 days

For ipickup = 1 To 7
If ipickup = 1 Then pickup = 0.000001
If ipickup = 2 Then pickup = 0.00001
If ipickup = 3 Then pickup = 0.0001
If ipickup = 4 Then pickup = 0.001
If ipickup = 5 Then pickup = 0.005
If ipickup = 6 Then pickup = 0.01
If ipickup = 7 Then pickup = 0.03
'Hosts ingest between 0.0001 and 3% of parasite larvae
```

```
initialconditions

year = 0
Do
year = year + 1

day = 0
Do
day = day + 1

calculate
deathinhost
pickupparasite
spreadparasite
freelivingdie
eggsdie
diegrouse
diechick
mature
If (day = repday) Then reproduce
If (day = independenceday) Then independence

cmdtimeday.Caption = "Day = " & day
cmdtimeyear.Caption = "Year = " & year
cmdAdults.Caption = "Adults = " & totaladults(day)
cmdrep.Caption = "Rep = " & rep
currente = eggs(day)
currentw = freeliving(day)

Loop Until (day = 365) Or (currentg >= maxg) Or (currentg <= mingrouse) Or
(totalchicks(day) >= maxchicks) Or violation = 1
Loop Until (year = maxyear) Or (currentg >= maxg) Or (currentg <= mingrouse) Or
(totalchicks(day) >= maxchicks) Or violation = 1
If (year = maxyear) Then finish = 1
If (currentg >= maxg) Then finish = 2
If (currentg <= mingrouse) Then finish = 3
If (totalchicks(day) >= maxchicks) Then finish = 4
If violation = 1 Then finish = 5

wfile

Next ipickup
Next iprobdiee
Next iprobdiew
Next iprobadnatural
Next iprobcd
Next irate
Next rep
cmdQuit.Caption = "Finished"
End Sub
```

APPENDIX 2

Visual Basic program code for the individual-based spatial territorial population model described in chapter 5.

Notes explaining sections of code are preceded by an apostrophe

```
Option Explicit
'INDIVIDUAL-BASED MODEL OF RED GROUSE AND T. TENUIS
'INCLUDES SPATIAL AND TERRITORIAL ASPECTS

'Initial conditions and fixed parameters
Const startG As Integer = 100                  'starting number of grouse
Const startP As Integer = 100                  'starting no. parasites per grouse
Const startW As Integer = 0                    'starting no. larvae in each grid cell
Const startE As Integer = 0                    'starting no. eggs in each grid cell
Const maxyear As Integer = 50                  'maximum number of years
Const repday As Integer = 238                  'day grouse reproduce
Const independenceday As Integer = 305         ' day chick become adults
Const fecund As Integer = 8                    'no. eggs per host
Const pfecund As Integer = 130                 'parasite fecundity
Const probdinhost As Single = 0.003            'adult parasite mortality
Const maxg As Integer = 30000                  'maximum number of grouse
Const mingrouse As Integer = 0                 'minimum number of grouse
Const maxchicks = 50000                        'maximum number of chicks
Const maxpinhost As Integer = 30000            'maximum parasites in one grouse
Const n As Integer = 50                        'cells of x and y grid
Const infectgrouse As Integer = 100            'no of grouse initially infected
Const Wongrid As Integer = 0                   'no. of grid cells infected with larvae
Const Eongrid As Integer = 0                   'no. of grid cells infected with eggs
Const critW As Integer = 10                    'min. no. larvae in 1 cell before extinctiont
Const critE As Integer = 10                    'min. no. eggs in 1 cell before extinction
Const probextinct As Single = 1                'probability of extinction
Const probmovegrouse As Single = 0.5           'probability of a grouse moving
Const stopmoveday As Integer = 150             'day when grouse stop moving
Const startmoveday As Integer = 258            'day when grouse start moving
Const territoryday As Integer = 150            'day when grouse form territories
Const near As Integer = 1                      'no. cells between territorial grouse

'Variable parameters
Dim probcd As Single          'proportion of chicks dying - not parasite induced
Dim probadnatural As Single 'proportion of adult grouse dying - not parasite induced
Dim probdiew As Single        'proportiong of free-living parasite larvae dying
Dim probdiee As Single        'proportion of parasite eggs dying
Dim pickup As Single          'proportion of larvae host ingests
Dim rate As Single            'proportion of parasite eggs developing to larvae
```

```
'Other variables
'c=counter
Dim rep As Integer 'no. of reps of the program
Dim iprobcd As Single 'c
Dim iprobadnatural As Single          'c
Dim iprobdiew As Single               'c
Dim iprobdiee As Single               'c
Dim ipickup As Single                 'c
Dim irate As Single                   'c
Dim repeattest As Integer             'c

Dim territory(1 To maxg) As Integer   'territory per adult grouse
Dim nchick(1 To maxg) As Integer      'no. chicks per adult grouse
Dim alive(1 To maxg) As Integer       c
Dim pingrouse(1 To maxg) As Single    'no parasites per adult grouse
Dim pinchick(1 To maxg) As Single     'no parasites per chick
Dim currentg As Integer               'c
Dim year As Integer                   'c
Dim day As Integer                    'c
Dim currentw As Single                'c
Dim currente As Single                'c
Dim totaladults(1 To 365) As Single   'no. adult grouse per day
Dim totalchicks(1 To 365) As Single   'no. chicks per day
Dim totalparasites(1 To 365) As Single 'no. parasites per day
Dim freeliving(1 To 365) As Single    'no. parasite larvae per day
Dim eggs(1 To 365) As Single          'no. parasite eggs per day
Dim finish As Integer                 'c
Dim violation As Integer              'c
Dim ott As Integer                    'c

Dim x(1 To maxg) As Integer           'x coordinate for each grouse
Dim y(1 To maxg) As Integer           'y coordinate for each grouse
Dim occupied(1 To n, 1 To n) As Integer 'grid cell occupied by grouse(1) or not (0)
Dim wonheather(1 To n, 1 To n) As Single 'no. parasite larvae in a cell
Dim eonheather(1 To n, 1 To n) As Single 'no. parasite eggs in a cell
Dim eggsfromhost As Single            'no. eggs produced by each grouse

Sub initialconditions() 'initial model conditions
Dim g%
violation = 0
ott = 0
currentg = startG

For g = 1 To currentg
nchick(g) = 0
pinchick(g) = 0
Next g

End Sub 'initialconditions
```

```
Sub distributegrouse() 'position each grouse in space
Dim i%, j%, g%

For i = 1 To n      'grid of n squares, all unoccupied
For j = 1 To n
occupied(i, j) = 0
Next j
Next i

For g = 1 To currentg
x(g) = Int((Rnd * n) + 1) 'x and y coordinates = random integers between 1 and n
y(g) = Int((Rnd * n) + 1)

'grouse are allowed to occupy the same space when they are distributed

occupied(x(g), y(g)) = 1
Next g
End Sub 'distributegrouse


Sub distributeadultparasite() 'infect each grouse with adult parasites
Dim g%, a%, ok%

For g = 1 To currentg
pingrouse(g) = 0 'all grouse uninfected
Next g

For g = 1 To infectgrouse 'infectgrouse is no. of grouse to be infected
Do
a = (Int((Rnd * currentg) + 1))
Loop Until pingrouse(a) = 0
pingrouse(a) = startP
Next g

End Sub 'distributeadultparasite


Sub distributeparasitelarvae() 'position parasite larvae in space
Dim i%, j%, w%, a%, b%
'in this version simulation starts with 0 freeliving parasites

For i = 1 To n
For j = 1 To n
wonheather(i, j) = 0
Next j
Next i

For w = 1 To Wongrid
a = (Int(Rnd * n) + 1)
b = (Int(Rnd * n) + 1)
```

```
wonheather(a, b) = wonheather(a, b) + startW
Next w

End Sub 'distributeparasitelarvae

Sub distributeparasiteeggs() 'position parasite eggs in space
Dim e%, i%, j%, a%, b%
'in this version simulation starts with 0 parasite eggs

For i = 1 To n
For j = 1 To n
eonheather(i, j) = 0
Next j
Next i

For e = 1 To Eongrid
a = (Int(Rnd * n) + 1)
b = (Int(Rnd * n) + 1)
eonheather(a, b) = eonheather(a, b) + startE
Next e

End Sub 'distributeparasiteeggs


Sub calculate() 'calculation of population numbers
Dim g%, i%, j%

totaladults(day) = currentg
totalchicks(day) = 0
totalparasites(day) = 0

For g = 1 To currentg
If nchick(g) = 0 Then totalparasites(day) = totalparasites(day) + pingrouse(g)
If nchick(g) > 0 Then
totalparasites(day) = totalparasites(day) + pingrouse(g) + (nchick(g) * pinchick(g))
totalchicks(day) = totalchicks(day) + nchick(g)
If (totalchicks(day) > maxchicks) Then totalchicks(day) = maxchicks And ott = 1
End If
Next g

freeliving(day) = 0
eggs(day) = 0

For i = 1 To n
For j = 1 To n
freeliving(day) = freeliving(day) + wonheather(i, j)
eggs(day) = eonheather(i, j) + eggs(day)
Next j
Next i
```

```
End Sub 'calculate


Sub wfile() 'data output file
Dim g%, totalday%

If rep = 1 Then
If repeattest = 1 And ipickup = 1 And iprobdiee = 1 And iprobdiew = 1 And
iprobadnatural = 1 And iprobcd = 1 And irate = 1 Then Open "c:\Ruth\territory1.txt"
For Output As #1
If repeattest > 1 Or ipickup > 1 Or iprobdiee > 1 Or iprobdiew > 1 Or iprobadnatural >
1 Or iprobcd > 1 Or irate > 1 Then Open "c:\Ruth\territory1.txt" For Append As #1
End If

If rep = 2 Then
If ipickup = 1 And iprobdiee = 1 And iprobdiew = 1 And iprobadnatural = 1 And
iprobcd = 1 And irate = 1 Then Open "c:\Ruth\territory2.txt" For Output As #1
If ipickup > 1 Or iprobdiee > 1 Or iprobdiew > 1 Or iprobadnatural > 1 Or iprobcd > 1
Or irate > 1 Then Open "c:\Ruth\territory2.txt" For Append As #1
End If

If rep = 3 Then
If ipickup = 1 And iprobdiee = 1 And iprobdiew = 1 And iprobadnatural = 1 And
iprobcd = 1 And irate = 1 Then Open "c:\Ruth\territory3.txt" For Output As #1
If ipickup > 1 Or iprobdiee > 1 Or iprobdiew > 1 Or iprobadnatural > 1 Or iprobcd > 1
Or irate > 1 Then Open "c:\Ruth\territory3.txt" For Append As #1
End If

If rep = 4 Then
If ipickup = 1 And iprobdiee = 1 And iprobdiew = 1 And iprobadnatural = 1 And
iprobcd = 1 And irate = 1 Then Open "c:\Ruth\territory4.txt" For Output As #1
If ipickup > 1 Or iprobdiee > 1 Or iprobdiew > 1 Or iprobadnatural > 1 Or iprobcd > 1
Or irate > 1 Then Open "c:\Ruth\territory4.txt" For Append As #1
End If

If rep = 5 Then
If ipickup = 1 And iprobdiee = 1 And iprobdiew = 1 And iprobadnatural = 1 And
iprobcd = 1 And irate = 1 Then Open "c:\Ruth\territory5.txt" For Output As #1
If ipickup > 1 Or iprobdiee > 1 Or iprobdiew > 1 Or iprobadnatural > 1 Or iprobcd > 1
Or irate > 1 Then Open "c:\Ruth\territory5.txt" For Append As #1
End If


If repeattest = 1 And rep = 1 And ipickup = 1 And iprobdiee = 1 And iprobdiew = 1
And iprobadnatural = 1 And iprobcd = 1 And irate = 1 Then
Print #1, "maxyear", maxyear
Print #1, "maxg", maxg
Print #1, "startg", startG
Print #1, "startp", startP
Print #1, "startw", startW
```

```
Print #1, "startE", startE
Print #1, "maxpinhost", maxpinhost
Print #1, "repday", repday
Print #1, "independenceday", independenceday
Print #1, "fecund", fecund
Print #1, "pfecund", pfecund
Print #1, "probdinhost", probdinhost

Print #1, " ", "finish", " ", "ott", "  ", "year", "  ", "day", "  ", "totaladults", "  ",
"totalchicks", "  ", "totalparasites", "  ", "freeliving", "  ", "eggs", "  ", "rate", " ",
"probcd", " ", " probadnatural", " ", "probdiew", " ", "probdiee", " ", "pickup"
End If

Print #1, finish, " ", ott, " ", year, "  ", day, "  ", totaladults(day), "  ", totalchicks(day),
" ", totalparasites(day), " ", freeliving(day), "  ", eggs(day), "  ", rate, "  ", probcd, "  ",
probadnatural, "  ", probdiew, "  ", probdiee, "  ", pickup

Close #1
```

End Sub 'wfile

Sub movegrouse() 'host random walk

```
Dim g%, i%, j%, newx%, newy%, randomv%, rx%, ry%, ok%

For g = 1 To currentg
newx = x(g)
newy = y(g)

If probmovegrouse > Rnd Then
Do
ok = 1
randomv = 0
rx = 0
ry = 0
randomv = Int(Rnd * 3) + 1  'random no. generation for change in x and y coordinates
If randomv = 1 Then rx = -1 'move left
If randomv = 2 Then rx = 0 'no move
If randomv = 3 Then rx = 1 'move right
randomv = Int(Rnd * 3) + 1
If randomv = 1 Then ry = -1 'move down
If randomv = 2 Then ry = 0 'no move
If randomv = 3 Then ry = 1 'move up

newx = x(g) + rx
newy = y(g) + ry

If (rx = 0) And (ry = 0) Then ok = 0 'grouse must move
If newx < 1 Then ok = 0 'grouse can't move off grid
If newx > n Then ok = 0
If newy < 1 Then ok = 0
```

```
If newy > n Then ok = 0
Loop Until (ok = 1)
End If
x(g) = newx
y(g) = newy
Next g

For i = 1 To n 'update occupied grid cells
For j = 1 To n
occupied(i, j) = 0
Next j
Next i

For g = 1 To currentg
occupied(x(g), y(g)) = 1
Next g
```

End Sub 'movegrouse

Sub deathinhost() 'adult parasite mortality

```
Dim g%

For g = 1 To currentg
pingrouse(g) = pingrouse(g) - (probdinhost * pingrouse(g))
If nchick(g) > 0 Then pinchick(g) = (pinchick(g) - (probdinhost * pinchick(g)))
Next g
```

End Sub 'deathinhost

Sub maketerritory() 'Grouse become territorial, only in territorial model

```
Dim g%, i%, j%, r%, d%, gdead%, noterritory%

For g = 1 To currentg
territory(g) = 0
alive(g) = 1
Next g

Do 'Until noterritories = 0

Do 'until territory(g) = 0
g = Int(Rnd * currentg + 1) 'pick a random grouse
Loop Until territory(g) = 0 'find one without a territory

territory(g) = 1 'give grouse a territory
For d = 1 To currentg 'go through all grouse to find any within 'near' distance of the one
chosen randomly (above)
If (territory(d) = 0) And ((x(g) - x(d)) <= near) And ((x(g) - x(d)) >= (0 - near)) And
((y(g) - y(d)) <= near) And ((y(g) - y(d)) >= (0 - near)) And (g <> d) Then
territory(d) = -1 'grouse too near doesn't take territory
alive(d) = 0 'and dies
```

```
pingrouse(d) = 0
nchick(d) = 0
pinchick(d) = 0
End If
Next d

noterritory = 0 'update no. grouse with and without territories
For g = 1 To currentg
If territory(g) = 0 Then noterritory = noterritory + 1
Next g

Loop Until noterritory = 0 '

gdead = 0 'calculate no. dead grouse
For g = 1 To currentg
If territory(g) = -1 Then gdead = gdead + 1
Next g

For i = 1 To n 'update grouse coordinates
For j = 1 To n
occupied(i, j) = 0
Next j
Next i

currentg = currentg - gdead

For g = 1 To currentg '
Do
r = r + 1
Loop Until alive(r) = 1 'find alive grouse
x(g) = x(r)
y(g) = y(r)
pingrouse(g) = pingrouse(r) 'update parasites in grouse
occupied(x(g), y(g)) = 1 'grouse occupies cell in grid as a territory
nchick(g) = nchick(r) 'update chicks with grouse
pinchick(g) = pinchick(r) 'update parasites in chicks
Next g
```

End Sub 'maketerritory

Sub pickupparasite() 'parasite ingestion

```
Dim g%, i%
Dim mark(1 To maxg) As Integer
Dim proportion As Single

For g = 1 To currentg
mark(g) = 0
Next g

For i = 1 To currentg
```

```
Do
g = Int(Rnd * currentg + 1)
Loop Until mark(g) = 0
mark(g) = 1

If nchick(g) = 0 Then
   pingrouse(g) = pingrouse(g) + (pickup * (wonheather(x(g), y(g))))
   wonheather(x(g), y(g)) = (wonheather(x(g), y(g)) - (pickup * wonheather(x(g),
y(g))))
End If

If nchick(g) > 0 Then

If (wonheather(x(g), y(g)) >= ((nchick(g) + 1) * pickup * wonheather(x(g), y(g)))) Then
   pingrouse(g) = pingrouse(g) + (pickup * (wonheather(x(g), y(g))))
   pinchick(g) = pinchick(g) + (pickup * wonheather(x(g), y(g)))
   wonheather(x(g), y(g)) = (wonheather(x(g), y(g)) - (pickup * (nchick(g) + 1) *
wonheather(x(g), y(g))))
End If

If (wonheather(x(g), y(g)) < ((nchick(g) + 1) * pickup * wonheather(x(g), y(g)))) Then
   proportion = wonheather(x(g), y(g)) / (1 + nchick(g))
   pingrouse(g) = pingrouse(g) + (proportion * (wonheather(x(g), y(g))))
   pinchick(g) = pinchick(g) + (proportion * wonheather(x(g), y(g)))
   wonheather(x(g), y(g)) = 0 'update free living parasite
End If

If pinchick(g) > maxpinhost Then pinchick(g) = maxpinhost
End If 'nchick(g)>0

If pingrouse(g) > maxpinhost Then pingrouse(g) = maxpinhost
Next i
```

End Sub 'pickupparasite

Sub spreadparasite() 'parasite reproduction

```
Dim g%

For g = 1 To currentg
eggsfromhost = 0
If nchick(g) = 0 Then
eggsfromhost = (pingrouse(g) * pfecund)
eonheather(x(g), y(g)) = eonheather(x(g), y(g)) + eggsfromhost 'add eggs to grid cell
End If
If (nchick(g) > 0) Then
eggsfromhost = (pingrouse(g) * pfecund) + (nchick(g) * pinchick(g) * pfecund)
eonheather(x(g), y(g)) = eonheather(x(g), y(g)) + eggsfromhost
End If
Next g
```

```
End Sub 'spreadparasite


Sub freelivingdie() 'parasite larval mortality
Dim i%, j%
Dim alllarvae!

alllarvae = 0
For i = 1 To n
For j = 1 To n
wonheather(i, j) = wonheather(i, j) - (probdiew * wonheather(i, j))
'parasite larvae die if they reach a critical low value
If (wonheather(i, j) < critW) And (probextinct > Rnd) Then wonheather(i, j) = 0
alllarvae = alllarvae + wonheather(i, j)
Next j
Next i
freeliving(day) = alllarvae

End Sub 'freelivingdie

Sub eggsdie() 'parasite egg mortality
Dim i%, j%
Dim alleggs!

For i = 1 To n
For j = 1 To n
eonheather(i, j) = eonheather(i, j) - probdiee * eonheather(i, j)
'parasite eggs die if they reach a critical low value
If (eonheather(i, j) < critE) And (probextinct > Rnd) Then eonheather(i, j) = 0
alleggs = alleggs + eonheather(i, j)
Next j
Next i
eggs(day) = alleggs

End Sub 'eggsdie

Sub diegrouse() 'grouse mortality
Dim ndead%, g%, r%, i%, j%

ndead = 0
For g = 1 To currentg
alive(g) = 1
Next g

For g = 1 To currentg
If (probadnatural > Rnd) Or ((pingrouse(g) / maxpinhost) > Rnd) Then
alive(g) = 0
ndead = ndead + 1
End If
If (alive(g) = 0) Then pingrouse(g) = 0
```

```
If nchick(g) > 0 And (alive(g) = 0) Then
nchick(g) = 0
pinchick(g) = 0
End If
Next g

For i = 1 To n 'update occupied grid cells
For j = 1 To n
occupied(i, j) = 0
Next j
Next i

'update grouse, parasites and chicks
r = 0
currentg = currentg - ndead

For g = 1 To currentg
Do
r = r + 1
Loop Until (alive(r) = 1)
x(g) = x(r) 'update position of grouse
y(g) = y(r)
pingrouse(g) = pingrouse(r) 'update parasites
occupied(x(g), y(g)) = 1
nchick(g) = nchick(r) 'update chicks
pinchick(g) = pinchick(r) 'update chick parasites
Next g
```

End Sub 'diegrouse

Sub diechick() 'chick mortality

```
Dim g%, i%, c%

For g = 1 To currentg
If nchick(g) > 0 Then
c = nchick(g)
For i = 1 To c
If probcd > Rnd Or ((pinchick(g) / maxpinhost) > Rnd) Then nchick(g) = nchick(g) - 1
Next i
End If
Next g
```

End Sub 'diechick

Sub mature() 'parasite egg development to larvae

```
Dim newlarvae!
Dim i%, j%

For i = 1 To n
For j = 1 To n
```

```
newlarvae = rate * eonheather(i, j)
wonheather(i, j) = wonheather(i, j) + newlarvae
eonheather(i, j) = eonheather(i, j) - newlarvae
eggs(day) = eggs(day) - newlarvae 'update total numbers of eggs
freeliving(day) = freeliving(day) + newlarvae 'update freeliving larvae
Next j
Next i
```

End Sub 'mature

Sub reproduce() 'grouse reproduction

```
Dim g%

For g = 1 To currentg
nchick(g) = fecund
pinchick(g) = 0
Next g
```

End Sub 'reproduce

Sub independence() 'chicks mature to adult grouse

```
Dim c%, g%, b%, nr&, i%

violation = 0
nr = 0 'count new recruits to adult population
For g = 1 To currentg
If nchick(g) > 0 Then
   For i = 1 To nchick(g)
   nr = nr + 1
   If (currentg + nr) > maxg Then violation = 1
   Next i
End If
Next g

If violation = 0 Then
c = 0
b = 0 'b is counter to add up all chicks present
For g = 1 To currentg
If nchick(g) > 0 Then
For b = (c + 1) To (c + nchick(g))
x(currentg + b) = x(g)
y(currentg + b) = y(g)
pingrouse(currentg + b) = pinchick(g)
Next b
c = c + nchick(g) 'adding total no. of mature chicks
End If '(nchick)
Next g
currentg = currentg + c 'add chicks to adult population
End If 'violation
```

```
For g = 1 To currentg
nchick(g) = 0 'update no. of chicks
pinchick(g) = 0 'update no. of parasites in chicks
Next g

End Sub 'independence

Sub update() 'calculations
Dim i%, j%, g%

For i = 1 To n
For j = 1 To n
occupied(i, j) = 0
Next j
Next i

For g = 1 To currentg
occupied(x(g), y(g)) = 1
Next g

End Sub 'update

Sub displayparasite() 'graphic representation of parasites
Dim g%, i%, j%
Dim wob%, hob%

wob = Picmoor.ScaleWidth / n
hob = Picmoor.ScaleHeight / n

For i = 1 To n
For j = 1 To n
   If wonheather(i, j) = 0 Then Picmoor.Line ((i - 1) * wob, (j - 1) * hob)-(i * wob, j *
hob), vbBlue, BF
   If eonheather(i, j) > 0 Then Picmoor.Line ((i - 1) * wob, (j - 1) * hob)-(i * wob, j *
hob), vbGreen, BF
   If wonheather(i, j) >= 10 Then Picmoor.Line ((i - 1) * wob, (j - 1) * hob)-(i * wob, j *
hob), vbWhite, BF
Next j
Next i

End Sub 'displayparasite

Sub displaygrouse() 'graphic representation of grouse
Dim g%, i%, j%
Dim wob%, hob%

wob = Picgrouse.ScaleWidth / n
hob = Picgrouse.ScaleHeight / n

For i = 1 To n
```

```
For j = 1 To n
If occupied(i, j) = 0 Then Picgrouse.Line ((i - 1) * wob, (j - 1) * hob)-(i * wob, j * hob),
vbBlue, BF
Next j
Next i

For g = 1 To currentg
   If (pingrouse(g) = 0) Then Picgrouse.Line ((x(g) - 1) * wob, (y(g) - 1) * hob)-(x(g) *
wob, y(g) * hob), vbRed, BF
   If (pingrouse(g) > 0) And (pingrouse(g) <= 100) Then Picgrouse.Line ((x(g) - 1) *
wob, (y(g) - 1) * hob)-(x(g) * wob, y(g) * hob), vbYellow, BF
   If (pingrouse(g) > 100) Then Picgrouse.Line ((x(g) - 1) * wob, (y(g) - 1) * hob)-(x(g)
* wob, y(g) * hob), vbMagenta, BF
   If (pingrouse(g) > 1000) Then Picgrouse.Line ((x(g) - 1) * wob, (y(g) - 1) * hob)-
(x(g) * wob, y(g) * hob), vbCyan, BF
Next g
```

End Sub 'displaygrouse

Private Sub cmdQuit_Click() 'quit button
End

End Sub 'cmdQuit

'START OF PROGRAM

```
Private Sub cmdStart_Click()

For rep = 1 To 10

Randomize

For irate = 1 To 3
If irate = 1 Then rate = 0.01
If irate = 2 Then rate = 0.028
If irate = 3 Then rate = 0.14

For iprobcd = 1 To 3
If iprobcd = 1 Then probcd = 0.002
If iprobcd = 2 Then probcd = 0.0085
If iprobcd = 3 Then probcd = 0.018

For iprobadnatural = 1 To 3
If iprobadnatural = 1 Then probadnatural = 0.0005
If iprobadnatural = 2 Then probadnatural = 0.0015
If iprobadnatural = 3 Then probadnatural = 0.0025

For iprobdiew = 1 To 7
If iprobdiew = 1 Then probdiew = 0.0005
```

```
If iprobdiew = 2 Then probdiew = 0.005
If iprobdiew = 3 Then probdiew = 0.02
If iprobdiew = 4 Then probdiew = 0.05
If iprobdiew = 5 Then probdiew = 0.1
If iprobdiew = 6 Then probdiew = 0.4
If iprobdiew = 7 Then probdiew = 0.8

For iprobdiee = 1 To 6
If iprobdiee = 1 Then probdiee = 0.005
If iprobdiee = 2 Then probdiee = 0.02
If iprobdiee = 3 Then probdiee = 0.05
If iprobdiee = 4 Then probdiee = 0.1
If iprobdiee = 5 Then probdiee = 0.4
If iprobdiee = 6 Then probdiee = 0.8

For ipickup = 1 To 4
If ipickup = 1 Then pickup = 0.00001
If ipickup = 2 Then pickup = 0.0001
If ipickup = 3 Then pickup = 0.001
If ipickup = 4 Then pickup = 0.01

initialconditions
distributegrouse
distributeadultparasite
distributeparasitelarvae
distributeparasiteeggs

year = 0
Do
year = year + 1

day = 0
Do
day = day + 1

calculate

If (day < stopmoveday) Or (day > startmoveday) Then
movegrouse
End If

If (day = territoryday) Then maketerritory
deathinhost
pickupparasite
spreadparasite
freelivingdie
eggsdie
diegrouse
dicchick
mature
```

```
If (day = repday) Then reproduce
If (day = independenceday) Then independence
update

cmdtimeday.Caption = "Day = " & day
cmdtimeyear.Caption = "Year = " & year
cmdAdults.Caption = "Adults = " & totaladults(day)
cmdrep.Caption = "rep = " & rep
displayparasite
displaygrouse

Loop Until (day = 365) Or (currentg >= maxg) Or (currentg <= mingrouse) Or
(totalchicks(day) >= maxchicks) Or violation = 1

Loop Until (year = maxyear) Or (currentg >= maxg) Or (currentg <= mingrouse) Or
(totalchicks(day) >= maxchicks) Or violation = 1
If (year = maxyear) Then finish = 1
If (currentg >= maxg) Then finish = 2
If (currentg <= mingrouse) Then finish = 3
If (totalchicks(day) >= maxchicks) Then finish = 4
If violation = 1 Then finish = 5

wfile

Next ipickup
Next iprobdiee
Next iprobdiew
Next iprobadnatural
Next iprobcd
Next irate
Next rep

cmdQuit.Caption = "Finished"

End Sub
```